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(57) Abstract

The present invention provides compositions and methods for the treatment of HIV infection. In particular, the present invention provides non–nucleoside inhibitors of reverse transcriptase (RT), as well as methods to treat HIV infection using these non–nucleoside inhibitors of RT. In preferred embodiments, the present invention provides a novel class of substituted benzimidazoles, effective in the inhibition of human immunodeficiency virus (HIV) RT.

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NON-NUCLEOSIDE INHIBITORS OF REVERSE TRANSCRIPTASE

FIELD OF THE INVENTION

The present invention is related to non-nucleoside inhibitors of reverse transcriptase (RT). In particular, the present invention relates to a novel class of substituted benzimidazoles effective in the inhibition of human immunodeficiency virus (HIV) RT.

BACKGROUND OF THE INVENTION

Since its recognition in 1981, the acquired immunodeficiency syndrome (AIDS) has become a major pandemic. The worldwide prevalence of HIV infection has been estimated at more than 18,500,000 cases, with an additional estimate of 1.5 million infected children (R. Famighetti, *1996 World Almanac and Book of Facts*, World Almanac Books, Mahwah, New Jersey, [1995], p. 840).

The etiologic agent associated with AIDS was identified as the human immunodeficiency virus (HIV). HIV is classified as a retrovirus, as it contains reverse transcriptase (RT), a multi-functional enzyme that contains RNA-dependent DNA polymerase activity, as well as DNA-dependent DNA polymerase and ribonuclease H activities. These three activities are essential for the conversion of genomic retroviral RNA into double-stranded DNA that can then be integrated into an infected host cell genome.

HIV is a D-type virus within the lentivirus family, with two major antigenic types (HIV-1 and HIV-2). HIV-1 and HIV-2 share approximately 40% genetic identity, although they can be readily distinguished based on differences in antibody reactivity to the envelope glycoprotein (M. Cloyd, "Human Retroviruses," *in* S. Baron (ed.), *Medical Microbiology*, University of Texas Medical Branch at Galveston, [1996], pp. 761-775). Both HIV-1 and HIV-2 have been associated with AIDS.

The search for effective drugs against HIV has focused on targeting various critical components of the replication cycle of HIV-1. One important component in this cycle is the reverse transcriptase enzyme. Indeed, perhaps because of its pivotal

role in the life cycle of HIV, it was the target of the first clinically approved anti-retroviral agents (*see*, Patel *et al.*, "Insights into DNA Polymerization Mechanisms from Structure and Function Analysis of HIV-1 Reverse Transcriptase," Biochem., 34:5351-5363 [1995]), although other compounds such as protease inhibitors have recently been introduced. In addition to its critical role in HIV replication, targeting RT has a potential benefit in reducing the toxicity to the patient associated with many drugs, as human cells do not normally contain this RT activity. Therefore, the potential for targeted inhibition of only viral replication, and not host cell multiplication is present. However, this potential has yet to be realized.

There are two major classes of RT inhibitors. The first comprises nucleoside analogues, such as 3'-azido-3'-deoxythymidine (AZT), 2',3'-didehydro-2',3'-dideoxythymidine (d4T), and 2',3'-dideoxycytidine (ddC). These compounds are analogs of normal deoxynucleoside triphosphates (dNTPs). However, these are not specific for HIV RT, and are incorporated into cellular DNA by host DNA polymerases, and can cause serious side effects. Moreover, administration of these analogs has resulted in the emergence of drug-resistant viral strains that contain mutations in their RT. Thus, these RT inhibitors have dangers that must be considered in developing treatment regimens for HIV-infected patients.

The second major class of RT inhibitors comprises the non-nucleoside RT inhibitors (NNRTI), such as tetrahydroimidazo(4,5,1-1-*jk*)(1,4)-benzodiazepin-2-(1*H*)-one, and -thione (TIBO) derivatives, dipyridodiazepinones, pyridinones, bis(heteroaryl)piperazines (BHAPs), 2',5'-bis-O-(tertbutyldimethylsilyl)-3'-spiro-5''-(4''-amino-1'',2''-oxathiole-2'',2''-dioxide)pyrimidine (TSAO) derivatives, α -anilinophenylacetamide (α -APA), 8-chloro-4,5,6,7-tetrahydro-5-methylimidazo-[4,5,1-jk][1,4]benzodiazepine-2 (1H)-one (8-Cl TIBO), and nevirapine. (*See*, *e.g.*, Pauwels *et al.*, "Potent and Selective Inhibition of HIV-1 Replication *in Vitro* By a Novel Series of TIBO Derivatives," Nature 343:470-474 [1990]; Merluzzi *et al.*, "Inhibition of HIV-1 Replication by a Non-Nucleoside Reverse Transcriptase Inhibitor," Science 250:1411-1413 [1990]; Goldman and Stern, "Pyridinone Derivatives: Specific Human

Immunodeficiency Virus Type 1 Reverse Transcriptase Inhibitors with Antiviral Activity," Proc. Natl. Acad. Sci. USA 88:6863-6867 [1991]; Romero and Tarpley, "Non-Nucleoside Reverse Transcriptase Inhibitors that Potently and Specifically Block Human Immunodeficiency Virus Type 1 Replication," Proc. Natl. Acad. Sci., USA 88:8806-8810 [1991]; Balzarini *et al.*, "2',3'-Bis-O-(Tertbutyldimethylsilyl)-3'-Spiro-5''-(4''-Amino-1'',2''-Oxathiole-2'',2''-Dioxide) Pyrimidine (TSAO) Nucleoside Analogs: Highly Selective Inhibitors of Human Immunodeficiency Virus Type 1 That are Targeted at the Viral Reverse Transcriptase," Proc. Natl. Acad. Sci. USA 89:4392-4396 [1992]; Young, "Non-Nucleoside Inhibitors of HIV-1 Reverse Transcriptase," Perspect. Drug Discov. Des., 1:181-192; and Pauwels *et al.*, "Potent and Highly Selective Human Immunodeficiency Virus Type 1 (HIV-1) Inhibition by a Series of α -Anilinophenylacetamide Derivatives Targeted at HIV-1 Reverse Transcriptase," Proc. Natl. Acad. Sci., USA 90:1711-1715 [1993]).

Unlike the nucleoside analogues, the NNRTIs do not act as chain terminators and do not bind at the dNTP-binding site. The majority of these compounds have been shown to share a common binding site unique to HIV-1 RT that is located in proximity to the RT polymerase active site. (*See*, Tantillo *et al.*, "Locations of Anti-AIDS Drug Binding Sites and Resistance Mutations in Three-Dimensional Structure of HIV-1 Reverse Transcriptase. Implications for Mechanisms of Drug Inhibition and Resistance," J. Mol. Biol., 243:369-387 [1994]; Smith *et al.*, "Molecular Modeling Studies of HIV-1 Reverse Transcriptase Nonnucleoside Inhibitors: Total Energy of Complexation as a Predictor of Drug Placement and Activity," Prot. Sci., 4:2203-2222 [1995]; Ding *et al.*, "Structure of HIV-1 TR/TIBO R86183 Complex Reveals Remarkable Similarity in the Binding of Diverse Nonnucleoside Inhibitors," Nature Struct. Biol., 2:407-415 [1995]; and Nanni *et al.*, "Review of HIV-1 Reverse Transcriptase Three Dimensional Structure: Implications for Drug Design," Perspect. Drug Discov. Des., 1:129-150 [1993]).

NNRTIs are highly specific for HIV-1 RT, and do not inhibit either HIV-2 RT or normal cellular polymerases, resulting in lower cytotoxicity and fewer side effects

than the nucleoside analogs. (*See, e.g.*, Ding *et al.*, "Structure of HIV-1 Reverse Transcriptase in a Complex with the Non-Nucleoside Inhibitor α-APA R 95845 at 2.8 Å Resolution," Structure 3:365-379 [1995]). However, resistance to some of these compounds has been reported. (*See, e.g.*, Nunberg *et al.*, "Viral Resistance to Human Immunodeficiency Virus Type 1-Specific Pyridinone Reverse Transcriptase Inhibitors," J. Virol., 65:4887-4892 [1991]; Tantillo *et al.*, "Locations of Anti-AIDS Drug Binding Sites and Resistance Mutations in the Three-Dimensional Structure of HIV-1 Reverse Transcriptase: Implications for Mechanisms of Drug Inhibition and Resistance," J. Mol. Biol., 243:369-387; and Richman, "Resistance of Clinical Isolates of Human Immunodeficiency Virus to Antiretroviral Agents," Antimicrob. Agents Chemother., 37:1207-1213 [1993]).

Despite recent developments in drug and compound design to combat HIV, there remains a need for a potent, non-toxic compound that is effective against wild type (WT) RTs, as well as RTs that have undergone mutations, and thereby become refractory to commonly used anti-HIV compounds.

SUMMARY OF THE INVENTION

The present invention is related to substituted benzimidazole compounds. In particular, the present invention provides non-nucleoside inhibitors of reverse transcriptase (RT) comprising a novel class of substituted benzimidazoles effective in the inhibition of human immunodeficiency virus (HIV) RT.

In one embodiment, the present invention provides 1-aryl-2-(2,6-difluorophenyl)-benzimidazole compositions with general structure of Figure 12. In particularly preferred embodiments, X is selected from the group consisting of H and methyl (CH_3). In other preferred embodiments, R'' is selected from the group consisting of 2,6-difluorobenzyl (2,6-F_2Bn), benzyl (Bn), 2,6-dichlorobenzyl (2,6-Cl_2Bn), 2,3,4,5,6-pentafluorobenzyl (2,3,4,5,6-F_5Bn), pyridylmethyl (CH_2(3-Py), benzenesulfonyl ($PhSO_2$), and 2,6-difluorophenylbenzoyl (2,6-F_2Bz). In yet other preferred embodiments, X is selected from the group consisting of H and methyl, and R'' is selected from the group consisting of 2,6-difluorobenzyl, benzyl, 2,6-

dichlorobenzyl, 2,3,4,5,6-pentafluorobenzyl, pyridylmethyl, benzenesulfonyl, and 2,6-difluorophenylbenzoyl.

In an alternative embodiment, the present invention provides 1-(2,6-difluorophenyl)-2-benzimidazole compositions with the general structure of Figure 13. In preferred embodiments, X' is selected from the group consisting of H and methyl (CH_3). In other preferred embodiments, R' is selected from the group consisting of phenyl (Ph), formyl (CHO), isopropyl (iPr), H, methyl (CH_3), hydroxymethyl (CH_2OH), and difluorobenzyloxymethyl ($CH_2O(2,6\text{-}F_2Bn)$), 2,6 diflourophenyl (2,6-F_2Ph), methylphenyl (2-CH_3Ph), pyridyl (*e.g.*, 4-Py, 3-Py), naphthyl (*e.g.*, 1-Nap, 2-Nap). In yet other particularly preferred embodiments, X' is selected from the group consisting of H and methyl, and R' is selected from the group consisting of phenyl, formyl, isopropyl, H, methyl, hydroxymethyl, and difluorobenzyloxymethyl.

In another alternative embodiment, the present invention provides 4,5,6, or 7-substituted-1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzamidazole compositions of the general structure of Figure 23, wherein X'''' is selected from the group consisting of H, methyl (CH_3), 4-methyl (4-CH_3), 5-methyl (5-CH_3), 6-methyl (6-CH_3), 7-methyl (7-CH_3), and 4,5-methyl (4,5-CH_3), 4-chloro (4-Cl), 5-chloro (5-Cl), 6-chloro (6-Cl), 4-bromo (4-Br), 5-bromo (5-Br), 4-nitro (4-NO_2), and 5-nitro (5-NO_2).

In yet another embodiment, the present invention provides 4-substituted-1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzaimidazole compositions of the general structure of Figure 24, wherein X''' is selected from the group consisting of methyl (CH_3), amine (NH_2), acetamide (NHAc), dimethylamine ($N(CH_3)_2$), bromine (Br), and chlorine (Cl).

It is contemplated that the substituted benzimidazoles of the present invention comprise derivatives containing various groups. It is not intended that the present invention be limited to particular substituted benzimidazole derivatives. For example, it is intended that the present invention encompass embodiments in which such groups as aromatic rings, hydrocarbons, and other structures are included. Such groups include, but are not limited to, 6-difluorobenzyl, benzyl, 2,6-dichlorobenzyl, 2,3,4,5,6-pentafluorobenzyl, pyridylmethyl, benzenesulfonyl, 2,6-difluorophenylbenzoyl, H

methyl, phenyl, formyl, isopropyl, H, methyl, hydroxymethyl, and difluorobenzyloxymethyl, 4-methyl, 5-methyl, 6-methyl, 7-methyl, and 4,5-methyl, methyl, amine, acetamide, dimethylamine, bromine, and chlorine. It is further intended that these groups be included in these compositions alone or in combination.

It is also contemplated that the aromatic residues of various embodiments of the present invention may be replaced with hydrophobic residues, such as aliphatic groups. For example, the present invention encompasses alkylimidazoles, including, but not limited to 1-(2,6-difluorobenzyl)-2-difluorophenyl-5,6-dialkylimidazole).

It is further contemplated that the present invention include embodiments in which the carbons (C) present on the benzyl ring (*i.e.*, C-4, C-5, C-6, and C-7) are replaced with nitrogen (N), singly, or in combination (*e.g.*, azapurines).

It is contemplated that the substituted benzimidazoles of the present invention will find use in treatment of HIV infection/disease. It particularly preferred embodiments, the present invention provides compositions of substituted benzimidazoles with activity against HIV-1 RT.

The present invention also provides methods for treatment of human immunodeficiency virus infection, comprising the steps of: providing: i) a subject suspected of being infected with human immunodeficiency virus; and ii) a composition having anti-reverse transcriptase activity, wherein the composition comprises at least one substituted benzimidazole with at least one substitution at the C-2 site, and at least one substitution at the N-1 site; exposing the subject to the composition; and observing for inhibition of anti-reverse transcriptase activity. In one preferred embodiment, the human immunodeficiency virus is HIV-1.

In one embodiment, the substituted benzimidazole is of the general structure of Figure 12. In an alternative embodiment, the substituted benzimidazole is of the general structure of Figure 13. In yet another embodiment, the substituted benzimidazole is of the general structure of Figure 23.

In addition, it is contemplated that the present invention encompass analogs of the benzimidazole ring system which undergo dissociation in the binding pocket of HIV RT, to give rise to electrophilic intermediates that react with nucleophilic sites in

the pocket. Thus, it is contemplated that compounds that act as irreversible inhibitors of HIV RT also be encompassed as embodiments within the present invention.

It is not intended that the compounds of the present invention be limited to any particular use. Indeed, it is intended that the compounds of the present invention will be utilized against organsims other than HIV.

DESCRIPTION OF THE FIGURES

Figure 1 shows the general structures of imidazole and benzimidazole.

Figure 2 is a schematic of the retrosynthetic analysis of N-benzyl-2-alkylbenzimidazoles by alkylation of 2-substituted benzimidazoles.

Figure 3 shows one embodiment for the synthesis of 2-aryl-benzimidazoles.

Figure 4 shows one embodiment for the synthesis of hydroxymethyl substituted benzimidazole.

Figure 5 shows the structure, as well as physical and biological data for 1-(2,6-difluorobenzyl)-2-aryl-benzimidazoles.

Figure 6 shows the structure, as well as physical and biological data for 1-aryl-2-(2,6-difluorophenyl)-benzimidazoles.

Figure 7 shows the structure, as well as physical and biological data for 1-(2,6-difluorobenzyl)-2-substituted-benzimidazoles.

Figure 8 shows the anti-RT activity of compounds **33** and **26**, compared with TBZ and TIBO.

Figure 9 shows the "butterfly-like" shape of TBZ and **39**.

Figure 10 shows the structure of four compounds that did not inhibit RT activity.

Figure 11 shows the substitutions of the benzimidazole core ring that inhibited as well as substitutions that did not inhibit HIV RT.

Figure 12 shows the structure of 1-aryl-2-(2,6-difluorophenyl)-benzimidazole.

Figure 13 shows the structure of 1-(2,6-difluorobenzyl)-2-benzimidazole.

Figure 14 shows one embodiment for the synthesis of substituted 1-(2,6-difluorobenzyl)-2-(-2,6-difluorophenyl)benzimidazoles.

Figure 15 shows one embodiment for the reduction of the 4-nitro group of **3100** with tin chloride.

Figure 16 shows the structure, physical, and enzyme inhibition data for 4, 5, 6 and 7-substituted-1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazoles (H, CH_3).

Figure 17 shows the structure, physical, and enzyme inhibition data for 4, 5, and 6-substituted 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazoles (Cl, Br, NO_2).

Figure 18 shows the structure, physical, and enzyme inhibition data for 4-substituted 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazoles.

Figure 19 shows the anti-RT activity of CH_3, NH_2, Cl, Br-substituted compounds, compared with TBZ and TIBO.

Figure 20 is a summary graph showing the cytotoxicity and anti-viral effect of compound **33**.

Figure 21 is a summary graph showing the cytotoxicity and anti-viral effect of compound **34**.

Figure 22 is a summary graph showing the anti-viral results for inactive compound **2100**.

Figure 23 shows the structure of 5,6, or 7-substituted-1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole.

Figure 24 shows the structure of 4-substituted 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole.

Figure 25 shows the calculated and actual purities of various substituted 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazoles.

DESCRIPTION OF THE INVENTION

The present invention provides substituted benzimidazole compounds, which act as non-nucleoside inhibitors of reverse transcriptase (RT). In particular, the present invention relates to a novel class of substituted benzimidazoles, effective in the inhibition of human immunodeficiency virus (HIV) RT.

Definitions

To facilitate understanding of the invention, a number of terms are defined below.

As used herein, the term "retrovirus" refers to the group of viruses with RNA genomes. Retroviruses are characterized has having reverse transcriptase, the enzyme that allows the RNA genome to be transcribed into DNA.

As used herein, the term "reverse transcriptase" refers to an enzyme with RNA-dependent DNA polymerase activity, with or without the usually associated DNA-dependent DNA polymerase and ribonuclease activity observed with wild-type reverse transcriptases.

As used herein, the term "anti-viral" is used in reference to any compound, substance, or molecule capable of inhibiting or preventing viral replication and/or dissemination. It is intended that the term encompass compounds capable of inhibiting viral replication by interfering with such activities as the reverse transcriptase activity of retroviruses. It is also intended to encompass "non-nucleoside reverse transcriptase inhibitors" (NNRTI). In preferred embodiments, the term is used in reference to substituted benzimidazole compounds.

As used herein, the term "chemotherapeutic" refers to any compound, element, or substance useful against disease. In preferred embodiments, the term encompasses compounds such as the substituted benzimidazoles of the present invention.

As used herein, the term "purified" refers to the removal of contaminants from a sample. Methods such as carbon, hydrogen and nitrogen analyses (CHN analysis, or "elemental analysis") may be used to determine the purity of compounds. In preferred embodiments, the CHN values of compounds of the present invention are very close to the predicted values. Correspondence of experimental with the predicted values to within 0.3% indicates high levels of purity. In particularly preferred embodiments, the compounds of the present invention have CHN values within 0.3% of the predicted values. In less preferred embodiments, the level of purity may be lower (*i.e.*, greater than 0.3% difference between the predicted and actual CHN values).

As used herein, the term "benzimidazole" is used in reference to molecules with the core structure as indicated in Figure 1. It is intended that the term encompass compounds in which substitutions, including additions, have been made to the chemical structure. The term encompasses, but is not limited to substitution reactions, wherein there is replacement of one or more atom or group in a molecule by another atom or group.

As used herein, the term "TBZ" refers to 1-(2,6-difluorophenyl)-1H,3-thiazolo[3,4-a]benzimidazole. In preferred embodiments, the present invention encompasses 1,2-substituted benzimidazoles, including but not limited to 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-4-methylbenzimidazole.

The term "cyclic compounds" refers to compounds having one (*i.e.*, a monocyclic compounds) or more than one (*i.e.*, polycyclic compounds) ring of atoms. The term is not limited to compounds with rings containing a particular number of atoms. While most cyclic compounds contain rings with five or six atoms, rings with other numbers of atoms (*e.g.*, three or four atoms) are also contemplated by the present invention. The identity of the atoms in the rings is not limited, though the atoms are usually predominantly carbon atoms. Generally speaking, the rings of polycyclic compounds are adjacent to one another; however, the term "polycyclic" compound includes those compounds containing multiple rings that are not adjacent to each other.

The term "heterocyclic compounds" refers broadly to cyclic compounds wherein one or more of the rings contains more than one type of atom. In general, carbon represents the predominant atom, while the other atoms include, for example, nitrogen, sulfur, and oxygen. Examples of heterocyclic compounds include benzimidazole, furan, pyrrole, thiophene, and pyridine.

The terms "aromatic," "aromatic compounds," and the like refer broadly to compounds with rings of atoms having delocalized electrons. The monocyclic compound benzene (C_6H_6) is a common aromatic compound. However, electron delocalization can occur over more than one adjacent ring (*e.g.*, naphthalene [two rings] and anthracene [three rings]). Different classes of aromatic compounds include,

but are not limited to, aromatic halides (aryl halides), aromatic heterocyclic compounds, aromatic hydrocarbons (arenes), and aromatic nitro compounds (aryl nitro compounds).

As used herein, the terms "aliphatic" and "aliphatic compounds" refer to compounds which comprise carbon atoms in chains, rather than the ring structure of aromatic compounds. It is intended that these aliphatic moieties will be bound to additional elements in some embodiments.

The terms "resistant" and "refractory" used in reference to "resistant mutants" of HIV and/or HIV RT, refer to the ability of some HIV RTs to function in the presence of compounds that are inhibitory to the RT of wild-type HIV. This resistance may result from any number of mutations, including but not limited to conformational changes in the RT structure, as well as the configuration of the RT bound to its substrate.

The term "mixture" refers to a mingling together of two or more substances without the occurrence of a reaction by which they would lose their individual properties. The term "solution" refers to a liquid mixture. The term "aqueous solution" refers to a solution that contains some water. In many instances, water serves as the diluent for solid substances to create a solution containing those substances. In other instances, solid substances are merely carried in the aqueous solution (*i.e.*, they are not dissolved therein). The term aqueous solution also refers to the combination of one or more other liquid substances with water to form a multi-component solution.

The terms "sample" and "specimen" in the present specification and claims are used in their broadest sense. On the one hand, they are meant to include a specimen or culture. On the other hand, they are meant to include both biological and environmental samples. These terms encompasses all types of samples obtained from humans and other animals, including but not limited to, body fluids such as urine, blood, fecal matter, cerebrospinal fluid (CSF), semen, and saliva, as well as solid tissue. These terms also refers to swabs and other sampling devices which are commonly used to obtain samples for culture of microorganisms.

Biological samples may be animal, including human, fluid or tissue, food products and ingredients such as dairy items, vegetables, meat and meat by-products, and waste. Environmental samples include environmental material such as surface matter, soil, water, and industrial samples, as well as samples obtained from food and dairy processing instruments, apparatus, equipment, disposable, and non-disposable items. These examples are not to be construed as limiting the sample types applicable to the present invention.

As used herein, the term "culture" refers to any sample or specimen which is suspected of containing one or more microorganisms. "Pure cultures" are cultures in which the organisms present are only of one strain of a particular genus and species. This is in contrast to "mixed cultures," which are cultures in which more than one genus, species, and/or strain of microorganism are present.

As used herein, the term "organism" is used to refer to any species or type of microorganism, including but not limited to viruses. In particular, the term is used in reference to RNA viruses, such as the retroviruses. In preferred embodiments, the organism of interest is HIV. In particularly preferred embodiments, the organism of interest is HIV-1.

The term "parenterally" refers to administration to a subject through some means other than through the gastrointestinal tract or the lungs. The most common mode of parenteral administration is intravenous. However, other modes of parenteral administration include, but are not limited to, intramuscular, and subcutaneous administration.

The phrase "pharmaceutical preparation suitable for parenteral administration" refers to a solution containing compound in a pharmaceutically acceptable form for parenteral administration. The characteristics of the form will depend on a number of factors, including the mode of administration. For example, a preparation for intravenous administration will often comprise compound dissolved in normal saline or sterile water for injection. Of course, the pharmaceutical preparations of the present invention are not limited to those diluents; indeed, other components or diluents known in the field of pharmaceuticals and pharmacy are within the scope of the present

invention. The pharmaceutical preparation may contain diluents, adjuvants and excipients, among other components, provided that those additional components neither adversely effect the preparation (*e.g.*, they do not cause degradation of the compound) nor the recipient (*e.g.*, they do not cause a hypersensitivity reaction).

Imidizoles And Benzimidazoles

In addition to compounds such as tetrahydroimidazo(4,5,1-1-*jk*)(1,4)-benzodiazepin-2-(1*H*)-one, and -thione (TIBO) derivatives, dipyridodiazepinones, pyridinones, bis(heteroaryl)piperazines (BHAPs), 2',5'-bis-O-(tertbutyldimethylsilyl)-3'-spiro-5''-(4''-amino-1'',2''-oxathiole-2'',2''-dioxide)pyrimidine (TSAO) derivatives, α -anilinophenylacemide (α -APA), 8-chloro-4,5,6,7-tetrahydro-5-methylimidazo-[4,5,1-jk][1,4]benzodiazepine-2 (1H)-one (8-Cl TIBO), and nevirapine, the potential therapeutic utility of imidazole compounds such as 1-(2,6-difluorophenyl)-1H,3H-thiazolo[3,4-a]benzimidazole (TBZ) has been shown. (*See, e.g.*, A. Chimirri *et al.*, "Anti-HIV Agents: Synthesis and In Vitro Anti-HIV Evaluation of Novel 1H,3H-Thiazolo[3,4-a]Benzimidazoles," Il Farmaco 46:817-823 [1991]).

As shown in Figure 1, imidazoles (*i.e.*, glyoxaline, 1,2-diazole, iminazole, miazole, pyrro[*b*]monazole, and 1,3-diaza-2,4-cyclopentadiene), are five-membered heterocycles with the formula $C_3H_4N_2$. The properties of these compounds permit the existence of cyclic aromatic structures (*i.e.*, derivatives) with more than two nitrogens or other heteroatoms bonded together. Many derivatives of these heterocyclic structures are important biochemical intermediates. Figure 1 also shows the structure of benzimidazole (*i.e.*, benziminazole, 1,3-benzodiazole, azindole, benzoglyoxaline, *N,N'*-methenyl-*o*-phenylenediamine, with the formula $C_7H_6N_2$). The numbering conventions for the ring positions are indicated in these structures.

Imidazoles (*e.g.*, clotrimazole, miconazole, econazole, and isoconazole) have found clinical use as anti-fungals, as they inhibit fungal cell ergosterol synthesis, but do not readily interfere with cholesterol synthesis in host (*e.g.*, mammalian) cells.

However, these drugs have undesirable side effects when administered systemically, such as pruritis, anemia, hyponatremia, leukopenia, thrombocytopenia, and elevated liver enzymes. Thus, their use has mainly been limited to topical treatment of fungal infections. Ketoconazole (another imidazole) is water-soluble and is easily absorbed from the gastrointestinal tract for oral treatment of systemic fungal infections. Although good results are usually obtained with otherwise healthy patients, severe problems in immunocompromised patients have been reported.

Benzimidazoles (*e.g.*, thiabendazole, mebendazole, and albendazole) have found clinical use as anti-helmintics, as they are effective against both the larval and adult stages of nematodes that cause ascariasis, intestinal capillariasis, enterobiasis, trichuriasis, as well as single and mixed hookworm infection. However, as with the imidazoles, the toxicity of these compounds, and/or their limited bioavailability has limited their clinical utility.

Benzimidazole derivatives have been investigated as anti-viral agents, and some have been recognized as being capable of inhibiting RNA viruses. (*See*, *e.g.*, Gilbert *et al.*, Antiviral Res., 9:355 [1988]). In addition, it has been recognized that thiazolobenzimidazole analogs may enhance the immune response. (*See*, *e.g.*, Warren *et al.*, Immunopharmacol., 1:269 ([1979]; Fenichel *et al.*, Immunopharmacol., 2:491 [1981]; and U.S. Patent No. 4,214,089 to Fenichel *et al.*, herein incorporated by reference). Other derivatives of thiazolobenzimidazole, such as 1-phenyl substituted 1H,3H-thiazole[3,4-a] benzimidazoles have also been reported as having anti-HIV-1 RT activity. (*See*, *e.g.*, EP 0471991, to Monforte *et al.*).

As mentioned above, one thiazolobenzimidazole compound, TBZ, has been shown to have HIV-1 RT inhibitory activity. However, there are some drawbacks to the use of TBZ. (*See*, *e.g.*, Chimirri *et al.*, Anti-HIV Agents. I. Synthesis and *In Vitro* Anti-HIV Evaluation of Novel 1H,3H-Thiazolo[3,4-a]Benzimidazoles," Il Farmaco 46:817-823 [1991]; Chimirri *et al.*, "Anti-HIV Agents. II. Synthesis and *In Vitro* Anti-HIV Evaluation of Novel 1H,3H-Thiazolo[3,4-1]Benzimidazoles," Il Farmaco 46:925-933 [1991]; and Buckheit *et al.*, "Thiazolobenzimidazole: Biological

and Biochemical Anti-Retroviral Activity of a New Non-Nucleoside Reverse Transcriptase Inhibitor," Antiviral Res., 21:247-265 [1993]). One problem with TBZ is its susceptibility to metabolic oxidation of the thiazolo ring, resulting in the formation of less potent sulfoxide and sulfone metabolites (El Dareer *et al.*, "Metabolism and Disposition of a Thiazolobenzimidazole Active Against Human Immunodeficiency Virus-1," Drug Metabol. Dispos., 21:231-235 [1993]).

Another problem is the loss of antiviral activity against HIV strains with mutated RT. (*See*, Boyer *et al.*, Analysis of Nonnucleoside Drug-Resistant Variants of Human Immunodeficiency Virus Type 1 Reverse Transcriptase," J. Virol., 67:2412-2420; and Buckheit *et al.*, "Comparative Anti-HIV Evaluation of Diverse HIV-1 Specific Reverse Transcriptase Inhibitor-Resistant Virus Isolates Demonstrates the Existence of Distinct Phenotypic Subgroups," Antiviral Res., 26:117-132 [1995]). During the development of the present invention, the drawbacks of TBZ were addressed in order to provide NNRTIs capable of efficiently and effectively inhibiting wild type, as well as mutated HIV-1 RT, with low toxicity levels, and a favorable therapeutic dose.

During early stages in the development of the present invention, retrosynthetic analysis was applied to TBZ. This indicated that opening the thiazolo ring of TBZ could result in the production of compounds potentially useful for inhibition of HIV RT, and resulted in the development of the novel benzimidazoles disclosed herein. Figure 2 shows a schematic for one embodiment of the present invention, in which N-benzyl-2-alkylbenzimidazoles are synthesized by alkylation of 2-substituted benzimidazoles, as was attempted during the development of the present invention. These substituted N-benzyl-benzimidazoles were of interest as potentially providing enhanced inhibition of wild type RT, and the various clinically observed variant forms of HIV RT. This was one highly important aspect of the present invention, as most of the known NNRTIs are rendered ineffective by the emergence of mutant forms of HIV. (*See*, De Clercq, "HIV Resistance to Reverse Transcriptase Inhibitors," Biochem. Pharm., 47:155-169 [1994]). Especially in view of the development of resistance to compounds previously effective against HIV, it was of great interest to develop

compounds effective against mutants of HIV RT. For example, development of a compound that was effective against Y181 C, a mutant that has a high degree of resistance to most NNRTIs, such as α -APA, nevirapine, and TIBO derivatives was a major consideration in the development of the present invention. (*See, e.g.*, "Structure of HIV-1 RT/TIBO R86183 Complex Reveals Similarity in the Binding of Diverse Nonnucleoside Inhibitors," Struct. Biol., 2:407-415 [1995]). Thus, during the development of the present invention, various compounds were developed and tested for their ability to inhibit both WT and mutated forms of HIV-1 RT. Various approaches were taken in order to produce these compounds, including synthesizing benzimidazoles with substitutions at one or more positions.

Synthesis Of Substituted Benzimidazoles

Figure 3 provide one embodiment of a general outline of the approach for the synthesis of 2-aryl-benzimidazoles. As shown in Figure 3, a variety of 2-aryl-benzimidazoles were made available by use of the appropriate choice of acylating reagent. In most cases, it was found that high yields of the desired N-acyl-nitroaniline could be obtained from either 2-nitroaniline (**8**), or 2-methyl-6-nitroaniline (**9**). In this Figure, "a" comprised aroyl chloride, pyridine/THF; "b" comprised Fe (**17**)/AcOH; "c" comprised 2,6-F_2-BnBr (**25**), NaH, THF; and "d" comprised BnBr (**27**) or $PhSO_2Cl$ (**44**) or 2,6-F_2BzCl (**7**), THF.

Only in the case of 1-naphthyl derivative (**14**) was a mixture of mono and bis acylated product formed. Subsequent reductive cyclization of compounds (**10-16**) with iron yielded the desired 2-aryl-benzimidazoles (**18-24**). Following their coupling with 2,6-difluorophenyl-α-bromotoluene (**25**), the desired 2-aryl-1-(2,6-difluorobenzyl)-benzimidazoles (R_2=2,6-difluorophenyl [**26** and **33**]; 2-methylphenyl [**36**]; napthyl [**37** and **38**]; pyridyl [**40** and **41**]) were obtained.

The first 2-substituted derivatives of N-2(2,6-difluorobenzyl)benzimidazole studied during the development of the present invention were the methyl, hydroxymethyl, isopropyl, carboxyl, formyl, and phenyl derivatives. With methyl and phenyl compounds, commercially available benzimidazoles were reacted with 2,6-

difluorophenyl-α-bromotoluene (**25**) to give compounds (**35** and **39**). Preparation of the hydroxymethyl substitute was achieved by acid-catalyzed condensation-cyclization of glycolic acid with either o-phenylenediamine (**1**) or 2,3-diaminotoluene (**3**), using an approach similar to that described by Chimirri *et al.* for the synthesis of TBZ. (Chimirri *et al.*, Anti-HIV Agents. I. Synthesis and *In Vitro* Anti-HIV Evaluation of Novel 1H,3H-Thizolo[3,4-a]Benzimidazoles," Il Farmaco 46:817-823 [1991]; and Chimirri *et al.*, "Anti-HIV Agents. II. Synthesis and *In Vitro* Anti-HIV Evaluation of Novel 1H,3H-Thiazolo[3,4-1]Benzimidazoles," Il Farmaco 46:925-933 [1991]). One embodiment, showing this approach is presented schematically in Figure 4. In this Figure, "a" comprised glycolic or isobutyric acid, 4 N HCl, reflux; "b" comprised 2,6-F_2-BzCl (7) or 2,6-F_2BnBr (**25**); "c" comprised *t*-butyldimethylsilylochloride (tBDMSCl), pyridine; "d" comprised Bu_4NF, THF; "e" comprised $KMnO_4$; and "f" comprised CrO_3.

In the embodiment presented in Figure 4, the hydroxymethyl was then protected with *t*-butyldimethylsilyl (TBDMS) before N-alkylation with 2,6-difluorophenyl-α-bromotoluene (**25**). Removal of TBDMS from (**31**) resulted in the production of 1-(2,6-difluorobenzyl)-2-hydroxymethyl-4-methylbenzimidazole (**49**).

However, oxidation of the hydroxymethyl to the carboxylic acid was found to be problematic. When a strong oxidant (*e.g.*, $KMnO_4$), the isolated product (**50**) indicated that decarboxylation occurred under acidic reaction conditions. Oxidation under basic conditions with chromium oxide similarly yielded (**50**), along with the formyl product (**51**). The carboxylic acid form was not isolated. A final product prepared from the 2-hydroxymethylbenzimidazole (**4**) was the bis-2,6-difluorobenzyl derivative (**32**).

The final series of compounds synthesized as described above, were analyzed in order to determine whether the position and nature of the substituents on the benzyl ring at N-1, were analogous to those in the TBZ series. In this series, the optimal anti-HIV activity was achieved when the phenyl ring was substituted at the 2 and 6 position with fluorine. Treatment of benzimidazole (**18** or **19**) with benzyl bromide

analogs (benzyl bromide itself, 2,6-dichloro-α-bromo-toluene, 2,3,4,5,6-pentafluoro-α-bromo-toluene), permitted the determination of whether compounds with hydrogen, chlorine, or multiple fluorines on N1 benzyl ring were better inhibitors of HIV-1 RT.

Since a number of NNRTI contain sulfonyl links (*e.g.*, 2-nitrophenyl phenyl sulfone, and 5-chloro-3-(phenylsufonyl)-indole-2-carboxamide), it was also determined whether a sulfonyl group could replace the methylene linker in compound (**33**). By reacting compound (**18**) or (**19**) with benzenesulfonyl chloride, compounds (**45**) and (**46**) were obtained in good yields. Similarly, treatment of compound **18** with 2,6-difluorobenzoyl chloride provided the N-2,6-difluorobenzoate (**47**), allowing the testing of carbonyl as a linker. In addition, the introduction of nitrogen into the N1-benzyl ring by synthesizing the 3-pyridyl derivative (**43**) via alkylation with α-bromo-methylpyridine was investigated.

Synthesis And Biological Activity of 4, 5, 6 And 7-Substituted Benzimidazoles

In addition to the 1,2-substituted benzimidazoles described above, 4, 5, 6, and 7 mono- and di-substitutions of 1-(2,6-difluorobenzyl)-2-(2,6-difluoropheny)benzimidazole, and its des-methyl analog (**26**) were synthesized and their properties observed. On the basis of inhibition of HIV-1 cytopathic effect (*i.e.*, protection from cell killing in the assay described in Example 75), it was determined that C-4 substituted analogs were consistently the most active compounds, as long as the substitution did not introduce strong electron withdrawing groups. Although it was less active, the 6-substituted analogs of **33** also exhibited desired activity. However, the 5 or 7-substituted analogues of **33** showed decreased RT inhibition.

Variation of the benzimidazole ring portion of **33** was accomplished mostly through determining and then using the appropriate choice of starting material. The general approach utilized in the synthesis of the desired substituted 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazoles is outlined in Figure 14. As regiocontrolled benzylation of C-2 substituted benzimidazoles was not expected for 5,6 or 7-substituted benzimidazoles, in order to control the regiochemistry, the N-acyl-nitro-anilines were alkylated before reductive cyclization. In Figure 14, "a" comprises

2,6-difluorobenzoyl chloride, pyridine:THF (1:1); "b" comprises NaOH, MeOH, dioxane; "c" comprises Br_2, pyridine, THF; "d" comprises 2,6-F_2BnBr, NaH, THF; and "e" comprises Fe, AcOH.

As indicated in Figure 14, it was determined that acylation of a number of substituted nitro-anilines with 2,6-difluorobenzoyl chloride (**7**) could yield the desired substituted N-(2,6-difluorobenzoyl)-nitro-anilines. In the case of the 4- or 5-chloro-nitro-anilines, the predominate product was however found to be the bis-N-acylated products, **400** and **600**. A variety of conditions involving time, temperature, the number of equivalents of reactants and the concentration were examined in order to identify suitable conditions for production of desired compounds. Unfortunately, appropriate conditions were not found that yielded the desired mono-acylated products, **500** and **700**. However, it was fortunately determined that the mono-N-acyl-nitro-aniline products could be obtained in high yields by selective de-acylation using NaOH in MeOH and dioxane. In most cases, alkylation of the N-acyl-nitro-aniline intermediates, **500** or **700** with 2,6-difluoro-α-bromo-toluene **1300** produced high yields of the desired N-(2,6-difluorobenzyl) products. The N-acyl-N-(2,6,-difluorobenzyl)nitro-anilines were subsequently reductively cyclized under similar conditions employed in the development of the 1 and 2-substituted compounds.

Synthesis of 1-(2,6-difluorobenzoyl)-4-bromo-nitroanilide **1200** was accomplished by bromination of 1-(2,6-difluorobenzoyl)-nitroanilide (**11**). Benzylation and reductive cyclization as done with the 4-chloro derivative was found to provide 5-bromo-1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole, **2100**, in good yields. Synthesis of 5-nitro-benzimidazole, **2900**, made use of 2-(2,6-difluorophenyl)-benzimidazole, as described above. Mono-nitration of 2-(2,6-difluorophenyl)benzimidazole with nitric acid at room temperature yielded 5-nitro-benzimidazole **2900** as the only product. It was subsequently determined that alkylation with 2,6-difluoro-α-bromo-toluene **1300** occurred regiospecifically to yield the 5-nitro-1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole, **3200**. Preparation of 4,5-dimethylbenzimidazole **2700** was accomplished starting from 2,3-

dimethyl-6-nitroaniline. Benzylation with **1300** was observed to occur regiospecifically due to the presence of 4-methyl group to yield **3000**.

Although an understanding of the mechanism is not necessary for the production and use of the present invention, mono acylation of 3-nitro-1,2-phenylenediamine with 2,6-difluorobenzoyl chloride (**7**), yielded a single product that was presumed to be the N-(2,6-difluorobenzoyl)-2-amino-3-nitroanilide, **800**, on the assumption that acylation occurred at the less hindered C-1 amino. Since cyclization of N-1 or N-2 acylated product would led to the desired 2-aryl-benzimidazole, detailed regiochemical analysis was not carried out. In contrast to all the previous reductive ring closures, cyclization of **800** was accomplished solely in the presence of refluxing acetic acid to yield 2-(2,6-difluorophenyl)-4-nitrobenzimidazole, **2800**. Alkylation of **2800** with 2,6-difluoro-α-bromo-toluene **1300** provided a key intermediate in the preparation of a number of 4-substituted benzimidazole derivatives.

As shown in Figure 15, reduction of the 4-nitro group of **3100** was accomplished with tin (II) chloride. In this Figure, "a" comprises 2,6-difluorobenzoyl chloride, pyridine: THF (1:1); "b" comprises AcOH reflux; "c" comprises 2,6-F_2-BnBr, NaH,THF; "d" comprises $SnCl_2$, AcOH, HCl; "e" comprises Ac_2O, THF; "f" comprises H_2CO, $NaBH_4$, H_2SO_4; and "g" comprises $NaNO_2$, HBr or HCl.

Compound **3300** was used to subsequently used to prepare the 4-bromo, **3400**, and 4-chloro, **3500**, benzimidazoles via Sandmeyer reactions (*i.e.*, methods known in the art). The 4-acetamido, **3600**, was prepared by mono-acylation of **3300** with acetic anhydride. Treatment of **3300** with formaldehyde and sodium borohydride yielded the dimethylamino compound, **3700**.

RT Inhibition By 1 And 2-Substituted Benzimidazoles

The testing of the 2-aryl-1-(2,6-difluorobenzyl)-benzimidazoles for their HIV-1 RT inhibition activity indicated that a number of aromatic systems were tolerated at the C2 position. These results are shown in Figure 5. In this Figure, the IC_{50}(μM) column indicates the quantity of drug required to reduce WT RT enzyme activity by 50% (IC_{50}).

As shown in Figure 6, the ability to inhibit wild type RT (WTRT) by the hydrogen (**50**), methyl (**35**), hydroxymethyl (**49**), isopropyl (**34**), formyl (**51**), phenyl (**39**), and bis-2,6-difluorobenzyl (**32**) compounds was measured as the percent inhibition of nucleotide incorporation into an rC-dG template primer at 10 μmolar drug concentration. Except for compound **39**, where the C2 was phenyl, all the substituents at the 2 position failed to appreciably inhibit HIV-1 RT. Substitution of the phenyl with fluorine at the 2 and 6 positions yielded the best inhibitor, compound **33** (IC_{50}=200 nM). The results included this Figure for 8-Cl-TIBO and TBZ are in contrast with those reported by Pauwels *et al.* (Pauwels *et al.*, "New Tetrahydroimidazo[4,5,1-jk][1,4]-Benzodiazepin-2(1H)-One and Thione Derivatives are Potent Inhibitors of Human Immunodeficiency Virus type 1 Replication and are Synergistic with 2',3'-Dideoxynucleoside Analogs," Antimicrob. Agents Chemother., 38:2863-2870 [1994]), who reported an IC_{50} for rC:dG of 0.06 μM for 8-Cl-TIBO, and Buckheit *et al.* (Buckheit *et al.*, "Thiazolobenzimidazole: Biological and Biochemical Anti-retroviral Activity of a New Nonnucleoside Reverse Transcriptase Inhibitor," Antiviral Res., 21:247-265 [1993]), who reported an IC_{50} for rC:dG of 0.5 μM for ribosomal RNA.

Although knowledge of the precise mechanism is not necessary to successfully practice the invention, it is contemplated that because the fluorines do not dramatically alter the size of the 2-phenyl ring, the four-fold increase in inhibitory activity observed from compounds **39** to **26** probably represented some alteration in the aromatic interactions between the 2,6-difluorophenyl ring, and the aromatic side chain residues surrounding the NNRTI binding pocket. Less conservative changes, such as the addition of an ortho-methyl to the 2-phenyl ring, led to a two-fold decrease in RT inhibition. (*See, e.g.*, **36**). This decrease in RT inhibition led to the examination of other planar aromatic systems. For example, changing the 2-phenyl to the 4-pyridyl resulted in almost no change in the IC_{50} value. (*See, e.g.*, compounds **39** and **41**). These results indicated that some heteroaromatic systems can be introduced at C2 without penalty. However, compound **40** (*i.e.*, the 3-pyridyl compound) showed

considerably less activity. While it is again not necessary to understand precise mechanisms in order to use the various embodiments of present invention, it is possible that the lone pair of electrons on the 4-pyridyl system can be accommodated, in contrast to the use of the 3-pyridyl compound, in which unfavorable interactions result. Larger aromatic moieties at C2 (*e.g.*, naphthyl), regardless of orientation, were found to result in complete loss of inhibitory activity, indicating that there was a limit to the size of the inhibitor the NNRTI binding pocket can accommodate. These observations led to the development of compounds with IC_{50}'s in the micromolar range, as shown in Figures 5 and 6.

As shown in the Table in Figure 6, the benezenesulfonyl and benzoate derivatives (**45**, **46**, and **47**) did not show appreciable RT inhibition activity. Substitution on the phenyl ring by a pyridine ring (**48**) similarly led to lower inhibition. Removal of the fluorine at the 2 and 6 position (**28** or **29**), or its replacement with chlorine (**30**) also resulted in a decrease in inhibition. Likewise, addition of more fluorines on the benzyl ring (**42**) yielded a compound showing greatly decreased inhibition activity.

Testing Of 4,5 And 5,6-Disubstituted And 7-Mono-Substituted Benzimidazoles Against Reverse Transcriptase

Testing of the 4,5 and 5,6 the 7-substituted benzimidazoles was determined by measuring the relative nucleotide incorporation using an rC-dG template primer at 1 mmol drug concentration (10-fold lower than used above), to the amount of incorporation with no inhibitor present utilizing WT HIV-1 RT. As seen in Figure 16, most of the methyl derivatives inhibited RT activity in this enzyme based assay. In these tests, the enzyme assay was conducted with WT RT. Although it is not necessary to understand precise mechanisms in order to use the present invention, a change in % inhibition was noted as the methyl group is moved from the 4 to 7 positions. In the case of the 5- and 7-methyl derivatives, the % inhibition is dramatically decreased from the 4- and 6-methyl derivatives. The observed decrease determined for the 7-methyl might be due to the adoption of a different conformation

from the 4-methyl owing to steric interactions between the 7-methyl and the N-1 benzyl group. Since the 5-methyl derivative, **2400**, can assume similar conformations as the 4-methyl derivative, **100**, and the 6-methyl derivative, **2500**, the decrease in % inhibition determined for the 5-methyl compound presumably must be due to differences in inductive effects. In the case of **3000**, with 4,5-dimethyl groups, the significant inhibition found with **100** possessing a single methyl at C-4 was dramatically decreased by the presence of the C-5 methyl.

It is was also observed that substitution at C-5 led to consistently less inhibition no matter what substitutent was examined, as shown in Figure 17. In comparing the 4,5, and 6 chloro compounds, the 5-chloro shows approximately two-fold more activity than either the 4- or 6-chloro compounds, **3500** or **2300**. A similar decrease is found when comparing the 4-bromo, **3400**, with the 5-bromo, **2100**. Even though the total inhibition was decreased by the presence of a strong withdrawing substitutent; this trend was also evident with the 4-nitro, **3100**, and 5-nitro, **2900**. These data led to further examination of the substitution at the C-4 position.

As seen in Figure 18, most of the 4-substituted benzimidazoles synthesized were able to inhibit RT in an enzyme based assay. In this Figure, the IC_{50} is indicated as the quantity of drug required to reduce WT RT enzyme activity by 50%. The "% inhibition (1 Mμ)" column indicates the percent inhibition of wild-type RT produced by 1 μM of the test compound. As with Figure 19, this experiment included two known compounds, 8-Cl TIBO and TBZ, for comparison purposes.

Surprisingly, the different group at C-4 did not show dramatic differences in IC_{50}'s. In fact, no difference was determined between a weak electron withdrawing group such as (Br), compared with a strong electron donating groups (NH_2). Even more surprising was the small difference between the bromo and chloro substitutents which have different van der Waals radii. It is possible that the similar IC_{50}'s reflect adjustments of the nucleoside binding pocket to maximize interaction with both these derivatives. Only in the case of the larger, 4-dimethylamino did the IC_{50} decrease by two-fold from **100**. In addition, it was only when a strong electron withdrawing group

(NO_2) or a large substitutent (NHAc) was placed at C-4 that the percent of inhibition decreased significantly.

In depth biological evaluation of the RT inhibitory properties of the most active 4-substituted compounds was determined with cultured MT-4 cells infected with WT and HIV-1 variants containing amino acid substitutions in RT, as described below. Comparison of the 4-methyl, **100**, with the 4-amino, chloro and bromo revealed similar activity against the different RT variants examined in the cytopathic cell killing assay. For most of the NNRTIs, the different amino acid substitutions in RT did not dramatically decrease the inhibitory activity except in the case of K100E and V106A.

These particular amino acid substitutions apparently caused similar loss of inhibitory activity of 8-Cl TIBO and nevirapine. It was noted that in the case of the 4-amino derivative, **3300**, slightly different responses were observed with the nearby mutations at L100I and K103N. With the K103N amino acid substitution, the substitution of an asparagine for lysine might result in favorable hydrogen bonding between the 4-amino group on the NNRTI and this amino acid substitution and thus led to the greater inhibition observed. In contrast, the L100I amino acid substitution caused a three-fold decrease in inhibition for the 4-amino compound, that was not found with other NNRTIs. Since the 4-amino derivative **3300**, is the only NNRTI included in the Examples which is capable of hydrogen bonding, this decrease in inhibition possibly reflected a conformation change in the binding of this NNRTI in the nonnucleoside binding pocket, causing the 4-amino group to makes unfavorable electrostatic interactions. Again, although an understanding of precise mechanisms is not necessary for the successful practice of of the present invention, since the large 4-bromo NNRTI did not show this same decrease in inhibitory activity, the loss is presumably not due to van der Waals interactions.

In Vivo **Reverse Transcriptase Activity**

Two of the most active difluorophenyl compounds (**26** and **33**) were tested for their RT inhibitory properties with cultured MT-4 cells infected with RT-variant strains of HIV. (*See*, Yang *et al.*, "Characteristics of a Group of Nonnucleoside Reverse Transcriptase Inhibitors with Structural Diversity and Potent Anti-Human Immunodeficiency Virus Activity," Leukemia 9:S75-S85 [1995]).

As shown in Figure 6, comparison of the 4-methyl derivative **33**, and its des-methyl analog **26**, revealed a consistent 3 to 4-fold better in inhibition wby th the methylated compound **33**, among the different viral isolates examined. The increased inhibition observed for **33** suggested that additional hydrophobic substituents on the benzimidazole ring can significantly improve binding of this class of compounds to the NNRTI pocket residues. It was also noted that in the case of the Y188 to C mutation in RT, the largest difference in inhibition (*i.e.*, 5-fold) between the methyl **33** and des-methyl **26** was observed. Although an understanding of these results is not necessary practice the invention, these data suggested that the methyl group in **33** points in the general direction of Tyr 188, by providing enhanced hydrophobic interaction over its unmethyl analog, when this region of the binding pocket is formed by cysteine.

As shown in Figure 8, a three-fold increase in RT inhibition was achieved using two embodiments of the present invention. Figure 8 shows the cross-resistance profile with one set of NNRTI-resistant HIV isolates from the cytopathic cell killing assay described in Example 75. In this Figure, antiviral data are reported as the quantity of drug in μM required to reduce cell killing or virus production by 50% (EC_{50}). The three 4XAZT isolates included in this Figure represent AZT drug-resistant variants.

The EC_{50} against WT RT for **33**, is similar to the activity with 8-Cl TIBO, a NNTRI presently undergoing clinical trials as an anti-AIDS drug. However, the present invention provides significant advantages over drugs such as TBZ and 8-Cl TIBO, as it retains activity against HIV mutants that have lost sensitivity to these drugs (*e.g.*, the many 8 -Cl TIBO inactivating mutants, such as A98G, L100I, V179D, and Y188 isolates). In sum, compound **33** was found to possess the best overall

biological profile of this series. Thus, the substitution of an aryl group for the thiazolo ring of TBZ has resulted in a potent, new NNRTI, that does not contain the metabolically active sulfur. In addition, it is contemplated that compound **33** will have broad activity against both WT and variant RTs. Nonetheless, it is also contemplated that other substitutions or different attachment sites may result in further optimization of the compounds of the present invention.

Figure 19 shows the cross-resistance profile with NNRTI-resistant HIV isolates from the cytopathic cell killing assay described in Example 75, for CH_3, NH_2, Cl, and Br-substituted, as well as 8-CL TIBO, and nivirapine. The various HIV isolates tested are included in this figure, with "WT" corresponding to wild-type virus, and the other strains listed by their RT mutations. The three 4XAZT isolates included in this Figure represent AZT drug-resistant variants. The 4xAZT/L1001 isolate has a double mutation; one mutation confers resistance to AZT, and the other mutation is located within the NNRTI binding pocket. In this Figure, antiviral data are reported as the quantity of drug in μM required to reduce cell killing or virus production by 50% (EC_{50}). 8-Cl TIBO and NVP (nevirapine) are two well-known NNRTIs, included in this experiment for comparison purposes.

In addition to Figures 8 and 19, which provide an indication of the profiles of various benzimidazoles against resistant HIV-1 isolates, Figures 20, 21, and 22 provide summary graph data for three compounds. Figure 20 shows the data for the cytotoxicity and anti-viral effect of compound **33**, while Figure 21 shows these data for compound **34**, and Figure 22 shows the results for inactive compound **2100**.

Geometry Of TBZ And 1-(2,6-Difluorobenzyl)-2-Phenylbenzimidazole

Semi-empirical quantum mechanical minimization at the AM1 level was used to compare the geometry of TBZ and 1-(2,6-difluorobenzyl)-2-phenylbenzimidazole (**39**). As shown in Figure 9, considerable similarity was observed between the energy minimized "butterfly-like" shapes of TBZ and **39**. For TBZ, the "butterfly-like" shape was previously determined by x-ray and NMR methods. (*See*, A. Chimirri *et al.*, "Thiazobenzimidazoles as Non-Nucleoside HIV-1 RT Inhibitors," Abst. II Congresso

Congiunto Italiano-Spagnolo di Chimica farmaceutica," Ferrara, Italy, August 30-September 3, 1995, ML20). For **39** in contrast to TBZ, more than one "butterfly-like" conformation can be adopted by rotation of the molecule's benzyl side chain. Although the C2 phenyl of the AM1 energy-minimized molecule **39** does not overlap the thiazolo ring of TBZ, at least two higher energy rotational isomers result in almost complete overlap. Although x-ray analysis is required to predict the correct "butterfly-like" orientation of this compound with RT and the NNRTI binding pocket, since some of the predominant contributions to the binding of NNRTI to RT involve π stacking and hydrophobic interactions. The extra aromatic ring present in **39** might significantly influence these interactions. This ability of HIV-1 RT to accommodate extra phenyl rings resulted in investigation of additional aromatic moieties.

Purity Of The Substituted Benzimidazoles

The active benzimidazole compounds of the present invention were produced at a very high purity level. As shown in Figures 25, the carbon, hydrogen and nitrogen analyses (CHN values) of these compounds were very close to the predicted values. CHN analysis (*i.e.*, elemental analysis) as known to those in the art, determines the amount of the elements in accurately weighed samples of the compound, and matches them against the amounts predicted from the elemental formulae. Correspondence of experimental with the predicted values to within 0.3% indicates high levels of purity.

EXPERIMENTAL

The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof. Although embodiments have been described with some particularity, many modifications and variations of the preferred embodiment are possible without deviating from the invention.

In the experimental disclosure which follows, the following abbreviations apply: PBS (phosphate buffered saline); MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide); EDTA (ethylenedinitrotetraacetic acid disodium salt);

HCl (hydrogen chloride); Tris (triphenylphosphine); NaCl (sodium chloride); SDS (sodium dodecyl sulfate); $Na_2S_2O_3$ (sodium thiosulfate); TAE (Tris-Acetate-EDTA); MeOH:CH_2Cl_2 (methanol:dichloromethane); H_2SO_4 (sulfuric acid); $FeSO_4$ (ferrous sulfate); $CuSO_4$ (cuprous sulfate); $MgSO_4$ (magnesium sulfate); NaOAc (sodium acetate); DMF (dimethyl formamide); THF (tetrahydrofuran); $NaHCO_3$ (sodium bicarbonate); HBr (hydrogen bromide); KBr (potassium bromide); DMSO (dimethyl sulfoxide); DMSO-d_6 (fully deuterated dimethyl sulfoxide); $CHCl_3$ (chloroform); $CDCl_3$ (deuterated chloroform); NH_3 (ammonia); Ph (phenyl; C_6H_5) Ac (ethanoate group); Et_2O (diethyl ether); EtOAc (ethyl acetate); CPE (cytopathic effect); ppm (parts per million); [α] (specific rotation); µL (microliters); µg (micrograms); mL (milliliters); L (liters); mg (milligrams); g (grams); hr or h (hours); mM (millimolar); M% (mole percent); µM (micromolar); nM (nanomolar); N (normal); nm (nanometers); min (minutes); mm (millimeter); kg (kilograms); δ (chemical shift); *J* or J (coupling constant); s (singlet); d (doublet); t (triplet); q (quartet); m (multiplet); vs (very strong); s (strong); m (medium); w (weak); vw (very weak); v (variable); mp (melting point); *c* (optical path length); NMR (Nuclear Magnetic Resonance); IR (Infrared Spectroscopy); MHz (megahertz); Hz (hertz); cm^{-1} (wavenumbers); eq (equivalents); M (Molar); µM (micromolar); N (Normal); mol (moles); mmol (millimoles); µmol (micromoles); nmol (nanomoles); g (grams); mg (milligrams); µg (micrograms); ng (nanograms); l or L (liters); ml (milliliters); µl (microliters); cm (centimeters); mm (millimeters); µm (micrometers); nm (nanometers); °C (degrees Centigrade); Ci (Curies); mCi (milliCuries); mp (melting point); TBZ (1-(2,6-difluorophenyl)-1H,3H-thiazolo[3,4-a]benzimidazole; RT (reverse transcriptase); WT (wild type); AZT (3'-azido-3'-deoxythymidine); NNRT (non-nucleoside reverse transcriptase); NNRTI (non-nucleoside reverse transcriptase inhibitors); BSA (bovine serum albumin); Et (ethyl); CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate); ddC (2',3'-dideoxycytidine); 8-Cl-TIBO (8-chloro-4,5,6,7-tetrahydro-5-methylimidazo[4,5,1-jk][1,4]benzodiazepin-2(1H)-one; THF (tetrahydrofuran); *t*- (tert);BDMSCl (*t*-butyldimethylsilylochloride); TBDMS (*t*-butyldimethylsilyl); IC_{50} (inhibitory concentration, 50%); EC_{50} (median effective

concentration); Varian (Varian Analytical Instruments, San Fernando, CA); Sigma (Sigma Chemical Co., St. Louis, MO); and (Aldrich Chemical Company, Inc., Milwaukee, WI).

Unless otherwise indicated, all chemicals and reagents were obtained from commercially available sources, such as Sigma and Aldrich. Where analyses are indicated by symbols of the elements, the observed results were within 0.4% of the theoretical values. Melting points were determined on an electrothermal apparatus using the supplied, stem-corrected thermometer and read, per methods known in the art. NMR spectra were recorded on a Varian 200 or 300 MHZ spectrometer, with Me_4Si as the internal standard. Merck silica gel (70-230 mesh and 230-400 mesh) were used for gravity and flash chromatography, respectively. Primes used in NMR assignments are defined by R' and R'' in the structures shown in Figure 3.

EXAMPLE 1

Preparation Of 2-Hydroxymethylbenzimidazole

In this Example, 2-hydroxymethylbenzimidazole (**2**) was prepared by stirring o-phenylenediamine (**1**)(1.3 g, 12 mmol), and 85% glycolic acid (2.74 g, 36 mmol, 300 M%), in 4 N HCL (40 mL), under reflux for 4 hours. After cooling to room temperature, the pH was adjusted to 7, with NaOH. The resulting crystals were filtered, washed with water and dried *in vacuo* (0.71 g, 4.8 mmol, 40% yield). ^{1}H-NMR (300 MHZ, DMSO-d_6): δ 7.48 (m, 2H, $H_{4,7}$), 7.13 (m, 2H,$H_{5,6}$), 5.67 (t, J=5.5 Hz, 1H, OH), 4.68 (d, J=5.5 Hz, 2H, CH_2).

EXAMPLE 2

Preparation Of 2-Hydroxymethyl-4-Methylbenzimidazole

In this Example, 2-hydroxymethyl-4-methylbenzimidazole (**4**) was prepared by stirring 2,3-diaminotoluene (**3**) (2.65 g 21.7 mmol), and 85% glycolic acid (8.20 g, 107.8 mmol, 500 M%) in 4 N HCl (80 mL), under reflux for 2 hours. After cooling to room temperature, the pH was adjusted to 7 with NaOH. The resulting brown precipitate was filtered, washed with water, and dried *in vacuo* (2.97 g, 18.3 mmol, 84% yield). ^{1}H-NMR (200 MHZ, DMSO-d_6): δ 7.28 (br d, 1H, H_7), 6.98 (dd, J=8.0, 7.3 Hz, 1H, H_6), 6.90 (m, 1H, H_5), 4.67 (s, 2H, CH_2), 2.49 (s, 3H, CH_3).

EXAMPLE 3

Preparation Of 2-(*t*-Butyldimethylsilyloxymethyl)-4-Methylbenzimidazole

In this Example, 2-(*t*-Butyldimethylsilyloxymethyl)-4-Methylbenzimidazole (**5**) was prepared by dissolving 2-hydroxymethyl-4-methylbenzimidazole (**4**) (1.50 g, 9.24 mmol) in pyridine (30 mL). To this mixture, *t*-BDMSCl (2.35 g, 15.6 mmol, 170 M%) was added. After 4 hours at room temperature, the reaction was concentrated to dryness. The residue was re-dissolved in CH_2Cl_2, and washed with $NaHCO_3$ (saturated aqueous), and NaCl (saturated aqueous), dried Na_2SO_4, filtered and concentrated. The product was purified by flash chromatography, eluting with 4% MeOH/CH_2Cl_2, and then re-crystallized from hexane (2.15 g, 7.78 mmol, 84% yield), to a white powder. ^{1}H-NMR (300 MHZ, CD_3OD): δ 7.36 (br d, J=11.1 Hz, 1H, H_7), 7.11 (dd, J=11.1, 10.4 Hz, 1H, H_6), 7.01 (br d, J=10.4 Hz, 1H, H_5), 4.94 (s, 2H, CH_2O), 2.55 (s, 3H, CH_3), 0.95 (s, 9H, Si*t*-Bu), 0.14 (s, 6H, $Si(CH_3)_2$).

EXAMPLE 4

Preparation Of 2-Isopropyl-4-Methylbenzimidazole

In this Example, 2-isopropyl-4-methylbenzimidazole (**6**) was prepared by dissolving 2,3-diaminotoluene (**3**) (1.00 g, 8.19 mmol), and isobutyric acid (4.0 mL, 43.1 mmol, 525 M%), in 4 N HCl (90 mL). After 2 hours at reflux, thee reaction was cooled in an ice bath, and the pH adjusted to 7 with NaOH. The resulting precipitate was filtered and washed with water. ^{1}H-NMR (200 MHZ, DMSO-d_6): δ 12.02 (br, 1H, NH), 7.26 (br, 1H, H_7), 6.99 (dd, J=7.4, 7.7 Hz, 1H, H_6), 6.87 (ddt, J=-0.8, 1.1, 7.4 Hz, 1H, H_5), 3.14 (sep, J=7.0 Hz, 1H, iPr), 2.48 (s, 3H, CH_3), 1.34 (d, J=7.0 Hz, 6H, iPr).

EXAMPLE 5

N-(2,6-Diflurobenzoyl)-2-Nitroanilide

In this Example, *N*-(2,6-diflurobenzoyl)-2-nitroanilide (**10**) was prepared using "Method A." The basic method of "Method A" was used in subsequent Examples, as indicated below, with reagent and other substitutions made as indicated.

First, 2-nitroaniline (**8**) (1.1 g, 8.0 mmol) was dissolved in THF (10 mL) and pyridine (2 mL). Then, 2,6-difluorobenzoyl chloride (**7**) (1.11 mL, 8.8 mmol, 110 M%) dissolved in THF (15 mL) was added to the first mixture. After stirring for 5 hours at room temperature, the reaction was concentrated to dryness. The residue was re-dissolved in ethylacetate, and washed with $NaHSO_4$ (5% solution), $NaHCO_3$ (saturated aqueous), and NaCl (saturated aqueous). The organic layer was dried under Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was recrystallized from ethylacetate/hexane, to produce crystals that were slightly yellow (1.7 g, 6.1 mmol, 76%), with a melting point of 139°C. ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 10.77 (s, 1H, NH), 8.89 (dd, J=1.2, 8.5 Hz, 1H, H_3), 8.26 (dd, J=1.5, 8.5 Hz, 1H, H_6), 7.75 (ddd, J=1.2, 7.9, 8.5 Hz, 1H, H_5), 7.50 (m, 1H, H_4), 7.29 (ddd, J=1.5, 7.9, 8.5 Hz, 1H, $H_{4)}$, 7.07 (m, 2H, $H_{3',5'}$).

EXAMPLE 6

Preparation Of *N*-(2,6-Difluorobenzoyl)-2-Methyl-6-Nitroanilide

In this Example, *N*-(2,6-difluorobenzoyl)-2-methyl-6-nitroanilide (**11**) was prepared using Method A. 2-methyl-6-nitroaniline (**9**) (2.25 g, 14.8 mmol) and 2,6-difluorobenzoyl chloride (**7**) (1.9 mL, 15 mmol, 100 M%) were mixed and stirred overnight. The solution was purified by gravity chromatography, and eluted with CH_2Cl_2/hexane/diethylether (380+120+10). The product was 2.36 g (8.1 mmol, 55% yield) of slightly yellow crystals. ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 8.55 (s, 1H, NH), 7.87 (dd, J=8.2, 0.92 Hz, 1H, H_5), 7.61 (d, J=7.4 Hz, 1H H_3), 7.48 (m, 1H, H_4), 7.38 (dd, J=7.4, 8.2 Hz, 1H, H_4), 7.05 (m, 2H, $H_{3',5'}$), 2.43 (s, 3H, CH_3).

EXAMPLE 7

Preparation Of *N*-Isonicotinoyl-2-Methyl-6-Nitroanilide

In this Example, *N*-isonicotinoyl-2-methyl-6-nitroanilide (**12**) was prepared using Method A. 2-methyl-6-nitroaniline (**9**) (1.52 g, 10.0 mmol), and isonicotinoyl chloride hydrochloride (2.95 g, 19.7 mmol, 200 M%) were stirred together for 4 hours. A second addition of isonicotinoyl chloride hydrochloride (1.05 g, 5.90 mmol, 60 M%) was added, and the solution was stirred overnight. The preparation was re-crystallized from diethylether:hexane (3:1) to produce 1.61 g (6.26 mmol, 53% yield) of white powder. ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 9.06 (br, 1H, NH), 8.82 (m, 2H, $H_{2',6'}$) 7.93 (br d, 7.9 Hz, H_6), 7.77 (m, 2H, H3'5'), 7.63 (br d, J=8.0 Hz, 1H, H_3), 7.40 (dd, J=7.9, 8.0 Hz, H_4).

EXAMPLE 8

Preparation Of *N*-(2-Methylbenzol)-2-Nitroanilide

In This Example, *N*-(2-methylbenzol)-2-nitroanilide (**13**) was prepared by Method A, by mixing 2-nitroaniline (**8**) (1.28 g, 9.3 mmol) and o-toluoyl chloride (1.52 mL, 11.6 mmol, 125 M%), and then recrystallized with ether:hexane (1:1) to produce 2.2 g (8.6 mmol, 92% yield) of yellow crystals of *N*-(2-methylbenzol)-2-nitroanilide. ^{1}H-NMR (300 MHZ, CD_2Cl_3): δ 10.75 (s, 1H, NH), 8.98 (d, J=8.5 Hz, 1H, H_3), 8.27 (d, J=8.5 Hz, H_6), 7.72 (dd, J=8.5, 7.4 Hz, 1H, H_5), 7.61 (d, J=8.4 Hz, 1H, H_6), 7.41 (dd, J=8.5, 7.4 Hz, 1H, H_4), 7.32 (t, J=7.4 Hz, 1H, H_4), 7.31 (d, J=7.4 Hz, 1H, H_3), 7.23 (dd, J=8.4, 7.4 Hz, 1H, H_5), 2.56 (s, 3H, CH_3).

EXAMPLE 9

Preparation Of *N*-(1-Naphthoyl)-2-Methyl-6-Nitroanilide

In this Example, *N*-(1-naphthoyl)-2-methyl-6-nitroanilide was prepared by Method A, by mixing 2-methyl-6-nitroaniline (**9**)(1.52 g, 10.0 mmol) and 1-naphthoyl chloride (2.00 mL, 13.3 mmol, 130 M%). After one hour of stirring at room temp, a second volume of 1-naphthoyl chloride (1.0 mL, 6.65 mmol, 66 M%) was added. The mixture was stirred for 6 hours and recrystallized with ethyl acetate to produce 2.79 g (9.11 mmol, 91% yield) of white powder. ^{1}H-NMR (200 MHZ, CD_2Cl_2): δ 8.09-7.99 (m, 2H), 7.77 (m, 1H), 7.66-7.41 (m, 5H), 7.23 (d, J=8.3 Hz, 1H), 7.24 (dd, J=7.3 Hz, 1H), 2.77 (s, 3H, CH_3).

EXAMPLE 10

Preparation Of *N*-(2-Naphthoyl)-2-Methyl-6-Nitroanilide

In this Example, *N*-(2-naphthoyl)-2-methyl-6-nitroanilide was prepared according to Method A, by mixing 2-methyl-6-nitroaniline (**9**)(1.52 g, 10.0 mmol) and 2-naphthoyl chloride (2.00 mL, 13.3 mmol, 130 M%). After 1 hour of stirring, a second volume of 2-naphthoyl chloride (1.0 mL, 6.65 mmol, 66 M%) was added. The mixture was stirred for 6 hours and recrystallized with ethyl acetate to produce 2.29 g (7.48 mmol, 75% yield) of white powder. ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 9.15 (br s, 1H, NH), 8.49 (s, 1H,$H_{1'}$), 8.06-7.86 (m, 5H, nap & H_5), 7.72-7.45 (m, 3H, nap & H_3), 7.37 (dd, J=7.73 Hz, 1H, H_4), 2.43 (s, 3H, CH_3).

EXAMPLE 11

Preparation Of *N*-Nicotinoyl-2-Methyl-6-Nitroanilide

In this Example, *N*-Nicotinoyl-2-methyl-6-nitroanilide (**16**) was prepared by Method A, by mixing 2-methyl-6-nitroaniline (**9**)(1.52 g, 10.0 mmol) and nicotinoyl chloride hydrochloride (2.67 g, 15.0 mmol, 150 M%). After stirring overnight and recrystallization from diethylether:hexane (3:1) 1.41 g (5.49 mmol, 55% yield) of white powder were produced. ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 9.16 (dd, J=0.8, 2.2 Hz, 1H, H_2), 9.01 (br, 1H, NH), 8.80 (dd, J=1.7, 4.8 Hz, 1H, H_6), 8.23 (ddd, J=1.7, 2.2, 8.0 Hz, 1H, H_4), 7.92 (m, 1H, H_5), 7.62 (br d, J=7.5 Hz, 1H, H_3), 7.48 (ddd, J=0.8, 4.8, 8.0 Hz, 1H, H_5), 7.39 (dd=7.5, 8.5 Hz, 1H, H_4), 2.39 (s, 3H, CH_3).

EXAMPLE 12

Preparation Of 2-(2,6-Difluorophenyl)benzimidazole

In this Example, 2-(2,6-difluorophenyl)benzimidazole (**18**) using Method B. The basic method of "Method B" was used in subsequent Examples, as indicated below, with reagent and other substitutions made as indicated.

First, 2-methyl-6-nitroaniline (**9**) (9.31 g, 31.9 mmol) was dissolved in glacial acetic acid (100 mL). Iron powder (**17**) (4.95 g) was then added. After 30 min at reflux, the reaction was concentrated to dryness, diluted with ethylacetate and washed with $NaHCO_3$. The aqueous layer was back extracted with ethylacetate and the combined organic solution was washed with $NaHCO_{3(sat\ aq)}$ and $NaCl_{(sat\ aq)}$, dried (Na_2SO_4), filtered and concentrated. The product was recrystallized from ethylacetate (6.24 g, 27.1 mmol, 85% yield of white powder). ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 9.92 (br, 1H, NH), 7.69 (br, 1H, $H_{4,7}$), 7.45 (m, 1H, H..), 7.31 (ddd, J=3.2, 4.0, 6.0 Hz, $H_{5,6}$), 7.11 (m, 2H, $H_{3',5'}$).

EXAMPLE 13

Preparation Of 2-(2,6-Difluorophenyl)-4-Methylbenzimidazole

In this Example, 2-(2,6-difluorophenyl)-4-methylbenzimidazole (**19**) was produced using Method B. *N*-(2,6-difluorobenzoyl)-2-methyl-6-nitroanilide (**11**) (1.35 g, 4.62 mmol) and iron powder (**17**) (1.3 g) mixed as described for Method B. After recrystallization from diethylether/hexane (3/1) 1.1 g (4.48 mmol, 97%) of colorless crystals were produced, with a melting point of 148°C. ^{1}H-NMR (300 MHZ, DMSO-d_6): δ 12.86 (s, 1H, NH), 7.66 (m, 1H, $H_{4'}$), 7.33 (m, 2H, $H_{3',5'}$), 7.27-7.18 (m, 1H, H_7), 7.15 (dd, J=8.0, 7.2 Hz, 1H, H_6), 7.05 (d, J=7.2 Hz, 1H, H_5), 2.55 (s, 3H, CH_3).

EXAMPLE 14

Preparation Of 2-(2-Methylphenyl)Benzimidazole

In this Example, 2-(2-methylphenyl)benzimidazole (**20**) was produced using Method B. *N*-(2-methylbenzol)-2-nitroanilide (**13**) (1.9 g, 7.4 mmol) and iron powder (**17**) (1.2 g) were mixed. After recrystallization from diethylether/hexane (3/1) 1.2 g (5.76 mmol, 78%) of colorless crystals were produced, with a melting point of 215°C. ^{1}H-NRM (300 MHZ, CD_2Cl_2): δ 7.63 (m, 1H, $H_{6'}$), 7.58 (dd, J=6.1, 3.2 Hz, 2H, $H_{4,7}$), 7.39-7.21 (m, 3H, $H_{3',4',5'}$), 7.25 (dd, J=6.1, 3.2 Hz, 2H, $H_{5,6}$), 2.58 (s, 3H, CH_3).

EXAMPLE 15

Preparation Of 2-(1-Napthyl)-4-Methylbenzimidazole

In this Example, 2-(1-napthyl)-4-methylbenzimidazole (**21**) was produced using Method B. The 1-naphthyl derivative shown as compound **14** (2.60 g, 11.7 mmol) and iron powder (**17**) (2.00 g), was purified by flash chromatography, eluting with 4% $MeOH/CH_2Cl_2$ and recrystallization from diethylether:hexane (3:1) 1.63 g (6.31 mmol, 54% yield) of white powder. ^{1}H-NMR (200 MHZ, CD_2Cl_2): δ 9.71 (br, 1H, NH), 8.80 (m, 1H), 8.01-7.89 (m, 2H), 7.81 (dd, J=1.3, 7.3 Hz, 1H), 7.61-7.47 (m, 4H), 7.21 (dd, J=7.3, 7.6 Hz, 1H), 7.11 (m, 1H), 2.66 (s, 3H, CH_3).

EXAMPLE 16

Preparation Of 2-(2-Napthyl)-4-Methylbenzimidazole

In this Example, 2-(2-napthyl)-4-methylbenzimidazole (**22**), using Method B. The compound shown as compound (**15**) (2.25 g, 7.34 mmol) and iron powder (**17**) (1.60 g) gave after purification by flash chromatography eluting with 4% $MeOH/CH_2Cl_2$ and recrystallization from diethylether:hexane (3:1), resulted in the production of 1.00 g (3.88 mmol, 53% yield) white powder. ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 8.70 (br, 1H, NH), 8.34 (dd, J=1.7, 8.6 Hz, 1H), 7.99-7.87 (m, 3H), 7.57-7.51 (m, 2H), 7.50-7.43 (m, 1H), 7.15 (dd, J=7.3, 7.5 Hz, 1H, H_6), 7.04 (m, 1H, H_5), 2.67 (s, 3H, CH_3).

EXAMPLE 17

Preparation Of 2-(3-Pyridyl)-4-Methylbenzimidazole

In this Example, 2-(3-pyridyl)-4-methylbenzimidazole (**23**) was produced using Method B. *N*-nicotinoyl-2-methyl-6-nitroanilide (**16**) (1.00 g, 3.89 mmol) and iron (**17**) (0.75 g) were mixed for 30 minutes. Additional iron powder (0.75 g) was then added, and the mixture prepared as described for Method B. Recrystallization from

ethylacetate resulted in the production of 0.72 g (3.44 mmol, 88% yield) of white powder; ^{1}H-NRM (300 MHZ, DMSO-d_6): δ 9.38 (dd, J=2.3, 0.9 Hz, 1H, $H_{2'}$), 8.67 (dd, J=1.7, 4.8 Hz, 1H, $H_{6'}$), 8.53 (ddd, J=1.7, 2.3, 8.0 Hz, 1H, $H_{4'}$), 7.58 (ddd, J=0.9, 4.8, 8.0 Hz, 1H, $H_{5'}$), 7.44 (dd, J=0.9, 8.1 Hz, H_7), 7.12 (dd, J=7.3, 8.1 Hz, 1H, H_6), 7.02 (ddt, J=0.2, 0.9, 7.3 Hz, 1H, H_5), 2.59 (s, 3H, CH_3).

EXAMPLE 18

Preparation Of 2-(4-Pyridyl)-4-Methylbenzimidazole

In this Example, 2-(4-pyridyl)-4-methylbenzimidazole (**24**) was produced using Method B. *N*-isonicotinoyl-2-methyl-6-nitroanilide (**12**)(1.02 g, 3.97 mmol) and iron powder (**17**) (1.10 g) were mixed as described for Method B. Recrystallization from ethylacetate resulted in the production of 0.70 g (3.34 mmol, 84% yield) of white powder; ^{1}H-NMR (300 MHZ, DMSO-d_6): δ 8.75 (m, 2H, $H_{2',6'}$), 8.14 (m, 2H, $H_{2',5'}$), 7.46 (dd, J=0.9, 8.1 Hz, H_7), 7.15 (dd, J=7.3, 8.1 Hz, 1H, H_6), 7.05 (ddt, J=0.8, 0.9, 7.3 Hz, 1H, H_5), 2.59 (s, 3H, CH_3).

EXAMPLE 19

Preparation Of 1-(2,6-Difluorobenzyl)-2-(2,6-Difluorophenyl)Benzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole (**26**) was prepared using Method C. The basic method of "Method C" was used in subsequent Examples, as indicated below, with reagent and other substitutions made as indicated.

The benzamidazole compound **18** (2.00 g, 8.70 mmol) and 2,6-difluoro-α-bromo-toluene (**25**) (2.85 g, 160 M%), were dissolved in THF (20 mL). To this mixture, NaH (0.75 g, 215 M%) was added. After mixing for 2 hours, the reaction was quenched with MeOH and concentrated. The residue was redissolved in ethylacetate, washed with $NaHCO_{3\,(sat\ aq)}$ and $NaCl_{\,(sat\ aq)}$, dried (Na_2SO_4), filtered and concentrated. The product was recrystallized from ethylacetate/hexane (1:1) (2.62 g,

0.35 mmol, 85% yield of white powder), mp 145°C. ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 7.77 (m, 1H, H_4), 7.54 (m, 1H, $H_{4'}$), 7.49'm, 1H, H7), 7.29 (m, 2H, $H_{5,6}$), 7.24 (m, 1H, $H_{4''}$), 7.08 (m, 2H, $H_{3',5'}$), 5.82 (m, 2H, $H_{3'',5''}$), 5.30 (s, 2H, CH_2PhF_2). Anal. ($C_{20}H_{12}F_4N_2$) C,H,N.

EXAMPLE 20

Preparation Of 1-Benzyl-2-(2,6-Difluorophenyl)Benzimidazole

In this Example, 1-benzyl-2-(2,6-difluorophenyl)benzimidazole (**28**) was produced using Method C. The benzamidazole compound **18** (100 mg, 0.43 mmol) and benzylbromide (**27**) (66.4 μl, 0.56 mmol) were mixed as indicated for Method C. After recrystallization from diethylether/hexane (3:1) 77 mg (0.24 mmol, 56%) of colorless crystals were produced, with a melting point of 124°C. ^{1}H-NMR (300 MHZ, $CDC1_3$): δ 7.88 (d, J=7.7 Hz, 1H, H_4), 7.47 (m, 1H, $H_{4'}$), 7.34-7.22 (m, 6H, $H_{5,6,7,2'',4'',6''}$), 7.07-6.99 (m, 4H, $H_{3',5',3'',5''}$), 5.28 (s, 2H, CH_2). Anal. ($C_{20}H_{14}F_2N_2x1/8H_2O$) C,H,N.

EXAMPLE 21

Preparation Of 1-Benzyl-2-(2,6-Difluorophenyl)-4-Methylbenzimidazole

In this Example, 1-benzyl-2-(2,6-difluorophenyl)-4-methylbenzimidazole (**29**) was produced according to Method C, using 2-(2,6-difluorophenyl)-4-methylbenzimidazole (**19**) (88mg, 0.33 mmol) and benzylbromide (**27**) (51 μL, 0.43 mmol) to produce, after recrystallization from diethylether/hexane (3:1), 67 mg (0.20 mmol, 61%) of colorless crystals, with a melting point of 112-117°C. ^{1}H-NMR (300 MHZ, $CDC1_3$): δ 7.46 (m, 1H, $H_{4'}$), 7.26-7.22 (m, 3H, $H_{7,3'',5''}$), 7.18-6.98 (m, 7H, $H_{5,6,3',5',2'',4'',6''}$), 5.25 (s, 2H, CH_2), 2.74 (s, 3H, CH_3). Anal. ($C_{21}H_{16}F_2N_2x1/4H_2O$) calcd. C,H,N 74.43, 4.91, 8.27; found C,H,N 74.81, 4.90, 7.85. HRMS 334.1281 (calcd) 334.1266 (found) δ ppm 4.6.

EXAMPLE 22

Preparation Of 1-(2,6-Dichlorobenzyl)-
2-(2,6-Diflouorophenyl)-4-Methylbenzimidazole

In this Example, 1-(2,6-dichlorobenzyl)-2-(2,6-diflouorophenyl)-4-methylbenzimidazole (**30**) was produced using Method C. In this Example, 2-(2,6-difluorophenyl)-4-methylbenzimidazole (**19**) (0.50 g, 2.05 mmol) and 2,6-dichloro-α-bromo-toluene (0.74 g, 3.08 mmol, 150 M%) were treated using Method C for 2 hours, purified by flash chromatography, eluted with 4% MeOH/CH_2Cl_2 and recrystallized from diethylether/hexane (3:1), to produce 0.70 g (1.74 mmol, 85% yield) of white powder; mp 202-203°C. ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 7.48 (m, 1H, H_4), 7.26 (m, 2H, $H_{5,7}$), 7.19 (dd, J=8.0, 8.2 Hz, 1H, H_6), 7.14-6.98 (m, 5H, $H_{3',5',3'',4'',5''}$), 5.56 (s, 2H, CH_2PhCl_2), 2.64 (s, 3H, CH_3). Anal. ($C_{21}H_{14}Cl_2F_2N_2x1/4H_2O$)C,H,N.

EXAMPLE 23

Preparation Of 1-(2,6-Difluorobenzyl)-
2-*t*-Butyldimethylsilyloxymethyl-4-Methylbenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-*t*-butyldimethylsilyloxymethyl-4-methylbenzimidazole (**31**) was produced using Method C. In this Example, 2-(*t*-Butyldimethylsilyloxymethyl)-4-Methylbenzimidazole (**5**) (3.25 g, 11.76 mmol) and 2,6-difluoro-α-bromo-toluene (**25**) (3.65 g, 150 M%) were treated according to Method C, for 4 h, and purified by flash chromatography with ethylacetate:hexane (1:4) to produce 4.41 g (10.96 mmol, 93% yield) of white powder. ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 7.32 (m, 1H, $H_{4''}$), 7.17 (br d, J=8.2 Hz, 1H, H_7), 7.08 (dd, J=7.3, 8.2 Hz, 1H, H_6), 7.00 (br d, J=7.3 Hz, 1H, H_5), 6.95 (m, 2H, $H_{3'',5''}$), 5.63 (s, 2H, CH_2PhF_2), 5.13 (s, 2H, CH_2O), 2.59 (s, 3H, CH_3), 0.94 (s, 9H, SitBu), 0.14 (s, 6H, $Si(CH_3)_2$).

EXAMPLE 24

Preparation Of 1-(2,6-Difluorobenzyl)-
2-(2,6-Difluorobenzyloxymethyl)Benzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(2,6-difluorobenzyloxymethyl) benzimidazole (**32**) was produced using Method C. In this Example, 2-hydroxymethylbenzimidazole (**2**) (92 mg, 0.62 mmol) and 2,6-difluoro-α-bromo-toluene (**25**) (334 mg, 1.61 mmol, 260 M%) were treated according to Method C. After recrystallization from diethylether/hexane (3:1), 66 mg (0.165 mmol, 27%) of colorless crystals were produced, with a melting point of 109°C. ^{1}H-NMR (300 MHZ, $CDCl_3$): δ 7.73 (m, 1H, H_4), 7.38 (m, 1H, H_7), 7.36-7.18 (m, 4H, $H_{5,6,4',4''}$), 6.98-6.82 (m, 4H, $H_{3',5',3'',5''}$), 5.58 (s, 2H, NCH_2PhF_2), 5.07 (s, 2H, OCH_2), 4.70 (s, 2H, OCH_2PhF_2). Anal. ($C_{22}H_{16}F_4N_2O$ x $1/2H_2O$)C,H,N.

EXAMPLE 25

Preparation Of 1-(2,6-Difluorobenzyl)-
2-(2,6-Difluorophenyl)-4-Methylbenzimidazole

In this Example, 1-(2,6-Difluorobenzyl)-2-(2,6-Difluorophenyl) -4-methylbenzimidazole (**33**) was produced using Method C. 2-(2,6-difluorophenyl)-4-methylbenzimidazole (**19**) (400 mg, 1.63 mmol) and 2,6-difluoro-α-bromo-toluene (**25**) (1.87 mmol, 388 mg) were treated according to Method C. After stirring overnight and recrystallization with diethylether/hexane (3:1), 453 mg (1.22 mmol, 75% yield) of white powder were produced, with a melting point of 182-186°C. ^{1}H-NMR (300 MHZ, $CDCl_3$): δ 7.48 (m, 1H, H_4), 7.32 (d, J=8.1 Hz, H_7), 7.20 (m, 1H, $H_{4''}$), 7.19 (dd, J=8.1, 7.2 Hz, 1H, H_6), 7.09 (d, J=7.2 Hz, 1H, H_5), 7.04 (m, 2H, $H_{3',5'}$), 6.79 (m, 2H, $H_{3'',5''}$), 5.33 (s, 2H, CH_2), 2.70 (s, 3H, CH_3). Anal ($C_{21}H_{14}F_4N_2$)C,H,N.

EXAMPLE 26

Preparation Of 1-(2,6-Difluorobenzyl)-2-Isopropyl-4-Methylbenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-isopropyl-4-methylbenzimidazole (**34**) was prepared according to Method C, using 2-isopropyl-4-methylbenzimidazole (**6**) (0.20 g 1.15 mmol) and 2,6-difluoro-α-bromo-toluene (**25**) (0.36 g, 1.74 mmol, 150 M%) stirred for 5 h, as described, purified by flash chromatography eluted with 4% MeOH:CH_2Cl_2 and recrystallized from diethylether:hexane (3:1) to yield 0.20 g (0.67 mmol, 58% yield) of white powder, with a melting point of 151-153°C. ^{1}H-NMR (200 MHZ CD_2Cl_2): δ 7.30 (m, 1H, $H_{4'}$), 7.15 (br d, J=7.7 Hz, 1H, H_7), 7.04 (dd, J=7.3, 7.7 Hz, 1H, H_6), 6.96 (br d, J=7.3 Hz, 1H, H_5), 6.82 (m, 2H, $H_{3',5'}$), 5.38 (s, 2H, CH_2PhF_2), 3.40 (sep, J=6.8 Hz, III, iPr), 2.57 (s, 3H, CH_3), 1.38 (d, J=6.8 Hz, 6H, iPr). Anal. ($C_{18}H_{18}F_2N_2$)C,H,N.

EXAMPLE 27

Preparation Of 1-(2,6-Difluorobenzyl)-2-Methylbenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-methylbenzimidazole (**35**) was prepared according to Method C, using 2-Methylbenzimidazole (204 mg, 1.54 mmol) and 2,6-difluoro-α-bromo-toluene (**25**) (351 mg, 1.70 mmol). Following recrystallization, from diethylether:hexane (3:1), 290 mg (1.12 mmol, 73%) of colorless crystals were produced, with a melting point of 99°C. ^{1}H-NMR (300 MHZ, $CDCl_3$): δ 7.66 (m, J=8.0, 1.1, 0.6 Hz, 1H, H_4), 7.37 (m, J=0.6, 1.1, 8.2 Hz, H_7), 7.30 (m, 1H, $H_{4'}$), 7.20 (m, J=8.0, 1.1, 7.3, H_5), 7.19 (m, J=8.2, 7.3, 1.1 Hz, 1H, H_6), 6.92 (m, 2H, $H_{3',5'}$) 5.35 (s, 2H, CH_2), 2.71 (s, 3H, CH_3). Anal. ($C_{15}H_{12}F_2N_2$)C,H,N.

EXAMPLE 28

Preparation Of 1-(2,6-Difluorobenzyl)-2-(2-Methylphenyl)Benzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(2-methylphenyl)benzimidazole (**36**) was produced according to Method C, using 2-(2-methylphenyl)benzimidazole (**20**) (0.10 g, 0.48 mmol) and 2,6-difluoro-α-bromo-toluene (**25**) (0.15 g, 0.73 mmol, 150 M%), purification by flash chromatography, diluted with 2% MeOH:CH_2Cl_2, and recrystallization with to produce 109 mg (0.33 mmol, 68%) of colorless crystals, with a melting point of 139°C. ^{1}H-NMR (300 MHZ, $CDCl_3$): δ 7.80 (m, 1H, H_4), 7.43-7.23 (m, 7H, $H_{5,6,7,3',4',5,6'}$), 7.21 (m, 1H, $H_{4''}$), 6.79 (m, 2H, $H_{3'',5''}$), 5.31 (s, 2H, CH_2), 2.23 (s, 3H, CH_3). Anal. ($C_{21}H_{16}F_2N_2$)C,H,N.

EXAMPLE 29

Preparation Of 1-(2,6-Difluorobenzyl)-2-(1-Napthyl)-4-Methylbenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(1-napthyl)-4-methylbenzimidazole (**37**) was produced according to Method C, using 2-(1-napthyl)-4-methylbenzimidazole (**21**) (0.30 g, 1.16 mmol) and 2,6-difluoro-α-bromo-toluene (**25**) (0.54 g, 2.60 mmol, 225 M%). After stirring overnight and purification by flash chromatography eluting with ethylacetate/hexane (1:4) and recrystallization from diethylether:hexane (3:1), 0.32 g (0.83 mmol, 72% yield) of white powder was produced, with a melting point of 121-123°C. ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 8.00 (d, J=8.1 Hz, 1H), 7.96 (d, J=8.1 Hz, 1H), 7.66 (d, J=6.3 Hz, 1H), 7.60 (t, J=7.5 Hz, 1H), 7.53 (dt, J=1.3, 7.5 Hz, 1H), 7.43 (dt, J=1.3, 7.6 Hz, 1H), 7.28 (d, J=8.1 Hz, 1H), 7.19 (t, J=7.5 Hz, 1H), 7.11 (m, 2H), 6.67 (m, 2H), 5.28 (s, 2H, CH_2PhF_2), 2.68 (s, 3H, CH_3). Anal. ($C_{25}H_{18}F_2N_2$) C,H,N.

EXAMPLE 30

Preparation Of 1-(2,6-Difluorobenzyl)-2-(2-Napthyl)-4-Methylbenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(2-napthyl)-4-methylbenzimidazole (**38**) was produced according to Method C, using 2-(2-napthyl)-4-methylbenzimidazole (**22**) (0.30 g, 1.16 mmol) and 2,6-difluoro-α-bromo-toluene (**25**) (0.36 g, 1.74 mmol, 150 M%), and a second addition of (25) (0.18 g, 0.87 mmol, 75 M%) after 2 hours of stirring. This mixture was stirred overnight, purified by flash chromatography, eluted with ethylacetate/hexane (1:4), and recrystallized from diethylether:hexane (3:1) to yield 0.35 g (0.91 mmol, 78% yield) of white powder, with a melting point of 175-176°C. ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 8.28(d, J=1.6 Hz, 1H), 8.02 (d, J=8.5 Hz, 1H), 7.96 (m, 2H), 7.83 (dd, J=1.7, 8.5 HZ, 1H), 7.59 (m, 2H), 7.20(m, 2H), 7.12 (m, 1H), 7:06 (m, 1H), 6.80 (m, 2H), 5.60 (s, 2H, CH_2PhF_2), 2.66 (s, 3H, CH_3). Anal. ($C_{25}H_{18}F_2N_2$) C,H,N.

EXAMPLE 31

Preparation Of 1-(2,6-Difluorobenzyl)-2-Phenylbenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-phenylbenzimidazole (**39**) was produced according to Method C, using 2-phenylbenzimidazole (300 mg, 1.54 mmol) and 2,6-difluoro-α-bromo-toluene (**25**) (1.70 mmol, 110 M%). Recrystallization from diethylether:hexane (3:1) yielded 300 mg (0.94 mmol, 63% yield) of colorless crystals, with a melting point of 163°C. ^{1}H-NMR (300 MHZ, $CDC1_3$): δ 7.82 (d, J=8.3 Hz, 1H, H_4), 7.75 (m, 2H, $2H_{3',5'}$), 7.53 (m, 3H, $H_{2',4',6'}$), 7.33 (d, J=8.3 Hz, 1H, H_7), 7.25 (m, 3H, $H_{5,6,4''}$), 6.81 (m, 2H, $H_{3'',5''}$), 5.55 (s, 2H, CH_2). Anal. ($C_{20}H_{14}F_2N_2x1/4H_2O$)C,H,N.

EXAMPLE 32

Preparation Of 1-(2,6-Difluorobenzyl)-2-(3-Pyridyl)-4-Methylbenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(3-pyridyl)-4-methylbenzimidazole (**40**) was produced according to Method C, using 2-(3-pyridyl)-4-methylbenzimidazole (**23**) (0:30 g, 1.43 mmol) and 2,6-difluoro-α-bromo-toluene (**25**) (0.49 g, 2.37 mmol, 165 M%). After stirring overnight, purification by flash chromatography eluting with 4% MeOH:CH_2Cl_2, and recrystallization from diethylether:hexane (3:1) 0.34 g (1.02 mmol, 71% yield) of white powder was produced, with a melting point of 186-188°C. ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 8.92 (dd, J=0.9, 2.3Hz, 1H, $H_{2'}$), 8.74 (dd, J=1.7, 4.9 Hz, 1H, H_6), 8.05 (dt, J=2.0, 7.8 Hz, 1H, $H_{4'}$), 7.48 (ddd, J=0.9, 4.9, 7.8, 1H, $H_{5'}$), 7.24 (m, 1H, $H_{4'}$), 7.22 (br d, J=7.7 Hz 1H, H_7), 7.15 (dd, J=7.7, 7.2, 1H, H_6), 7.07 (dt, J=1.0, 7.2 Hz 1H H_5), 6.83 (m, 2H, $H_{3',5'}$), 5.51 (s, 2H, CH_2PhF_2), 2.64 (s, 3H, CH_3). Anal. ($C_{20}H_{15}F_2N_3$)C,H,N.

EXAMPLE 33

Preparation Of 1-(2,6-Difluorobenzyl)-2-(4-Pyridyl)-4-Methylbenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(4-pyridyl)-4-methylbenzimidazole (**41**) was produced according to Method C, using 2-(4-pyridyl)-4-methylbenzimidazole (**24**) (0.30 g, 1.43 mmol) and 2,6-difluoro-α-bromo-toluene (**25**) (0.45 g, 2.17 mmol, 150 M%). After stirring overnight, purification by flash chromatography eluting with 4% MeOH:CH_2Cl_2, and recrystallization from diethylether:hexane (3:1), 0.29 g (0.86 mmol, 60% yield) of white powder was produced with a melting point of 171-172°C. ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 8.77 (dd, J=1.6, 4.4 Hz, 2H, $H_{2',6'}$), 7.66 (dt, J=1.4, 4.4 Hz, 2H, $H_{3',5'}$), 7.24 (m, 1H, $H_{4'}$), 7.22 (dd, J=0.8, 8.1 Hz, 1H, H_7), 7:15 (dd, J=7.5, 8.1 Hz, 1H, H_6), 7.07 (ddq, J=0.4, 0.8, 7.5 Hz, 1H, H_5), 6.82 (m, 2H, $H_{3',5'}$), 5.54 (s, 2H, CH_2PhF_2), 2.63 (s, 3H, CH_3). Anal. ($C_{20}H_{15}F_2N_3$)C,H,N.

EXAMPLE 34

Preparation Of 1-(2,3,4,5,6-Pentafluorobenzyl)-
2-(2,6-Difluorophenyl)-4-Methylbenzimidazole

In this Example, 1-(2,3,4,5,6-pentafluorobenzyl)-2-(2,6-difluorophenyl)-4-methylbenzimidazole (**42**) was prepared according to Method C, using 2-(2,6-difluorophenyl)-4-methylbenzimidazole (**19**) (0.31 g, 1.27 mmol) and 2,3,4,5,6-pentafluoro-α-bromo-toluene (0.30 mL, 1.99 mmol, 155 M%). After stirring for 4 hours, purification by flash chromatography, elution with 4% MeOh/CH_2Cl_2 and recrystallization from diethylether:hexane (3:1), 0.33 g (0.77 mmol, 61% yield) of white powder was produced, with a melting point of 155-156°C. ^{1}H-NMR (200 MHZ, CD_2Cl_2); δ 7.57 (m, 1H, H_6), 7.29-7.23 (m, 2H, $H_{6,7}$), 7.19-7.05 (m, 3H, $H_{5,3',5'}$), 5.35 (s, 2H, CH_2PhF_5), 2.64 (s, 3H, CH_3). Anal. ($C_{21}H_{11}F_7N_2$)C,H,N.

EXAMPLE 35

Preparation Of 1-(3-Pyridylmethyl)-
2-(2,6-Difluorophenyl)-4-Methylbenzimidazole

In this Example, 1-(3-pyridylmethyl)-2-(2,6-difluorophenyl)-4-methylbenzimidazole (**43**) was produced according to Method C, using 2-(2,6-difluorophenyl)-4-methylbenzimidazole (**19**) (0.21 g, 0.86 mmol) and α-bromo-methylpyridine (0.22 g, 1.28 mmol, 150 M%). After mixing for 1 hour, purification by flash chromatography eluting with ethylacetate/hexane (1:1) increasing to ethylacetate (100%), and recrystallization from diethyl ether:hexane (3:1), 0.24 g (0.73 mmol, 85% yield) of white powder were produced, with a melting point of 131-132°C. ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 8.45 (d, J=3.4 Hz, 1H, $H_{6''}$), 8.32 (s, 1H, $H_{2''}$), 7.53 (m, 1H, $H_{4''}$), 7.25-7.03 (m, 7H, $H_{5,6,7,3',5',4'',5''}$), 5.26 (s, 2H, CH_2Py), 2.67 (s, 3H, CH_3-Ar). Anal. ($C_{20}H_{15}F_2N_3x1/2H_2O$)C,H,N.

EXAMPLE 36

Preparation Of 1-Benzenesulfonyl-2-(2,6-Difluorophenyl)benzimidazole

In this Example, 1-benzenesulfonyl-2-(2,6-difluorophenyl)benzimidazole (**45**) was produced according to Method D. The basic method of "Method D" was used in subsequent Examples, as indicated below, with reagent and other substitutions made as indicated.

The benzamidazole compound **18** (0.31 g, 1.34 mmol) dissolved in THF (5 mL) was added to NaH (0.10 g, 190 M%). After 5 min, benzenesulfonyl chloride (**44**) (0.25 mL, 0.35 g, 2.00 mmol, 150 M%) was added. After stirring for 2 h, the reaction was dissolved in ethylacetate, washed with $NaHCO_{3\ (sat\ aq)}$ and $NaCl_{(sat\ aq)}$, dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 2% MeOH/CH_2Cl_2 and then recrystallized from diethylether:hexane (3:1), to yield (0.41 g, (1.10 mmol, 83% yield) of white powder, with a melting point of 104-106°C. ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 8.09 (m, 1H, $PhSO_2$), 7.77 (m, 1H, $PhSO_2$), 7.69 (m, 2H, $PhSO_2$), 7.66-7.53 (m, 2H, $H_{4,7}$), 7.51-7.39 (m, 4H, $PhSO_2$, $H_{3,5}$), 7.07 (m, 2h, $H_{3',5'}$). Anal. ($C_{19}H_{12}F_2N_2SO_2$) C, H, N.

EXAMPLE 37

Preparation Of 1-Benzenesulfonyl-
2-(2,6-Difluorophenyl)-4-Methylbenzimidazole

In this Example, 1-benzenesulfonyl-2-(2,6-difluorophenyl)-4-methylbenzimidazole (**46**) was produced according to Method D, using 2-(2,6-difluorophenyl)-4-methylbenzimidazole (**19**) (0.20 g, 0.82 mmol) and benzenesulfonyl chloride (**44**) (0.20 mL, 0.28 g. 1.58 mmol, 190 M%), purified by flash chromatography eluting with 2% MeOH/CH_2Cl_2 and recrystallized from diethyletherhexane (3:1), to produce 0.24 g (0.02 mmol, 76% yield) of white powder, with a melting point of 134-135°C. ^{1}H-NMR (200 MHZ, CD_2Cl_2): δ 7.89 (br d, J=

8.2 Hz, 1H), 7.73-7.38 (m, 6H), 7.34 (dd, J= 7.4, 8.1 Hz, 1H, H_6), 7.2 (br d, J= 7.4 Hz, 1H, H_5), 7.07 (m, 2H, $H_{3',5'}$), 2.59 (s, 3H, CH_3), Anal. ($C_{20}H_{14}F_2N_2SO_2$) C,H,N.

EXAMPLE 38

Preparation Of 1-(2,6-Difluorobenzoyl)-2-(2,6-Difluorophenyl)Benzimidazole

In this Example, 1-(2,6-difluorobenzoyl)-2-(2,6-difluorophenyl)benzimidazole (**47**) was produced according to Method D, using the benzamidazole compound **18** (30 mg, 0.13 mmol) dissolved in pyridine (0.5 mL) and chloroform (1.2 mL). To this mixture 2,6-difluorobenzoyl chloride (**7**) was added (20 µl; 0.16 mmol, 120 M%), and the mixture was stirred at room temperature for 5 h, diluted with chloroform, and washed with $NaHSO_{4\ (2\%\ solution)}$. The organic layer was dried (Na_2SO_4), filtered, and evaporated. The solid was recrystallized from diethylether/hexane to produce 19 mg of colorless crystals (40% yield), with a melting point of 145°C. ^{1}H-NMR (300 MHZ, $CDCl_3$): δ 8.14 (m, 1H, H_4), 7.89 (m, 1H, H_7), 7.48 (m, 2H, $H_{5,6}$), 7.31-7.18 (m, 2H, $H_{4',4''}$), 6.77 (m, 4H, $H_{3',5',3'',5''}$). Anal. ($C_{20}H_{10}F_4N_2O$) C,H,N.

EXAMPLE 39

Preparation Of 1-(2,6-Difluorobenzyl)-
2-Hydroxymethyl-4-Methylbenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-hydroxymethyl-4-methylbenzimidazole (**48**) was prepared according to Method D, using 1-(2,6-difluorobenzyl)-2-*t*-butyldimethylsilyloxymethyl-4-methylbenzimidazole (**31**) (1.82 g, 4.52 mmol) dissolved in THF (20mL). Tetrabutyl ammonium fluoride (1.45 g, 4.60 mmol, 100 M%) was then added. After 30 min at room temperature, the reaction was concentrated to dryness. The residue was suspended in water, filtered, and washed with water to produced 1.28 g, (4.44 mmol, 98% yield) of white powder. ^{1}H-NMR (300 MHZ, CD_3OD): δ 7.39(m, 1H, $H_{4''}$), 7.18 (br d, J= 8.3 Hz, 1H, H_7), 7.09 (dd, J=

7.4, 8.3 Hz, 1H, H6), 7.05-6.97 (m, 3H, $H_{3',5',5}$), 5.68 (s, 2H, CH_2PhF_2), 4.99 (s, 2H, CH_2O), 2.57 (s, 3H, CH_3). Anal. ($C_{16}H_{14}F_2N_2O$) C,H,N.

EXAMPLE 40

Preparation Of 1-(2,6-Difluorobenzyl)-4-Methylbenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-4-methylbenzimidazole (**49**), according to Method D, using 1-(2,6-difluorobenzyl)-2-hydroxymethyl-4-methylbenzimidazole (**48**) (1.82 g, 4.52 mmol) dissolved in 1.5 M H_2SO_4 (40 mL). $KMnO_4$ (1.50 g, 9.49 mmol, 160 M%) was then added. After 1 h at room temperature, the reaction mixture was filtered and washed with water. The brown solid was collected, suspended in acetone/methanol and filtered. The filtrate was collected and purified by flash chromatography eluting with 10% MeOH/CH_2Cl_2 increasing to 50% MeOH/CH_2Cl_2 to produce 1.42 g, (4.70 mmol, 80% yield) of white powder. ^{1}H-NMR (200 MHZ, CD_2Cl_2): δ 7.99 (br s, 1H, H_2), 7.37 (br d , J= 7.9 Hz, 1H, H_7), 7.32 (m, 1H, $H_{4'}$), 7.17 (dd, J= 7.3, 7.9 Hz, 1H, H_6), 7.03 (d, J= 7.3 Hz, 1H, H_5), 6.96 (m, 2H, $H_{3',5'}$), 5.40 (s, 2H, CH_2PhF_2), 2.59 (s, 3H, CH_3), Anal. ($C_{15}H_{12}F_2N_2$ x 1/4H_2O) C,H,N.

EXAMPLE 41

Preparation Of 1-(2,6-Difluorobenzyl)-2-Formyl-4-Methylbenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-formyl-4-methylbenzimidazole (**50**) was prepared according to Method D. Pyridine (3.4 mL) dissolved in CH_2Cl_2 (50 mL) was added to CrO_3 (2.20 g). After 15 min., 1-(2,6-difluorobenzyl)-2-hydroxymethyl-4-methylbenzimidazole (**48**) dissolved in DMF was added to the mixture and stirred. After 20 m, the organic solution was decanted from a tarry black deposit. The organic solution was washed with 5% NaOH, 5% HCl, $NaHCO_3$ and NaCl, dried (Na,SO_4), filtered, and concentrated. Purification by flash chromatography eluting with 2% MeOH/CH_2Cl_2 gave 0.55 g (1.92 mmol. 44% yield) of 1-(2,6-difluorobenzyl)-2-formyl-4-methylbenzimidazole (**50**) and 0.17 g (0.66 mmol, 15% yield) of 1-(2,6-

difluorobenzyl)-4-methylbenzimidazole (**49**). ^{1}H-NMR (200 MHZ, CD_3OD): δ 10.13 (s, 1H, CHO), 7.36-7.10 (cm, 4H, $H_{5,6,7,4'}$), 6.90 (m, 2H, $H_{3',5'}$), 6.05 (s, 7H, Ch_2PhF_2), 2.66 (s, 3H, CH_3). Anal. ($C_{16}H_{12}F_2N_2Ox1/5H_2O$)C,H,N.

EXAMPLE 42

Preparation Of *N,N*-Bis-(2,6-Difluorobenzoyl)-4-Chloro-2-Nitroanilide

This Example, and the following group of Examples (through Example 73) describe the synthesis of 4-substituted benzimidazoles.

In this Example, *N,N*-bis-(2,6-difluorobenzoyl)-4-chloro-2-nitroanilide (**400**) was prepared. First, 4-chloro-2-nitroaniline (1.05 g, 6.08 mmol) was dissolved in THF:pyridine (1:1) (20 mL), and 2,6-difluorobenzoyl chloride (**7**) was added (1.9 mL, 15.1 mmol, 250 M%). After 6 hours of mixing, a second aliquot of (**7**) (0.6 mL, 4.77 mmol, 78 M%). After stirring overnight at room temperature, the reaction was concentrated to dryness. The residue was redissolved in CH_2Cl_2, washed with $NaHCO_{3\,(sat.\ aq)}$ and $NaCl_{\,(sat.\ aq)}$, dried (Na_2SO_4), filtered and concentrated. The product was recrystallized from ethylacetate (2.67 g, 5.90 mmol, 97% yield) to produce white crystals). ^{1}H-NMR (300 MHZ, CD_2Cl_2): δ 8.15 (d, J= 2.4 Hz, 1H, H_3), 7.67 (dd, J= 2.4, 8.5 Hz, 1H, H_5), 7.51 (d, J= 8.5 Hz, 1H, H_6), 7.36 (m, 2H, $H_{4',4''}$), 6.89 (m, 4H, $H_{3',5',3'',5''}$).

EXAMPLE 43

Preparation Of *N*-(2,6-Difluorobenzoyl)-4-Chloro-2-Nitroanilide

In this Example, *N*-(2,6-difluorobenzoyl)-4-chloro-2-nitroanilide (**500**) was prepared. First, *N,N*-bis-(2,6-difluorobenzoyl)-4-chloro-2-nitroanilide (**400**) (1.00 g, 4.42 mmol) was dissolved in methanol/dioxane (1:1) (40 mL), and sodium hydroxide (0.27 g, 6.75 mmol, 150 M%) was then added to the mixture. After stirring for 30 minutes at room temperature, the reaction was quenched with $NaHSO_4$, diluted with

CH_2Cl_2, washed with $NaHCO_{3\,(sat\ aq)}$ and $NaCl_{\,(sat\ aq)}$, dried (Na_2SO_4), filtered and concentrated. The product (1.28 g, 4.09 mmol, 93 % yield) was recrystallized from ethylene oxide/hexane (3:1). ^{1}H-NMR (300 MHz, CD_2Cl_2): δ 10.72 (s, 1H, NH), 8.91 (d, J= 9.1 Hz, 1H, H_6), 8.27 (d, J= 2.5 Hz, 1H, H_3), 7.71 (dd, J= 9.1, 2.5 Hz, 1H, H_5), 7.52 (m, 1H, $H_{4'}$), 7.08 (m, 2H, $H_{3',5'}$).

EXAMPLE 44

Preparation Of *N,N*-Bis-(2,6-Difluorobenzoyl)-5-Chloro-2-Nitroanilide

In this Example, *N,N*-bis-(2,6-difluorobenzoyl)-5-chloro-2-nitroanilide (**600**) was prepared. First, 5-chloro-2-nitroaniline (1.02 g, 5.91 mmol) was dissolved in pyridine/THF (1:1) (20 mL), and 2,6-difluorobenzoyl chloride (**7**) (1.50 mL, 11.9 mmol, 200 M%) was then added. After stirring overnight at room temperature, the reaction was concentrated to dryness. The residue was redissolved in CH_2Cl_2, washed with $NaHCO_{3\,(sat.\ aq)}$ and $NaCl_{\,(sat.\ aq)}$, dried (Na_2SO_4), filtered, and concentrated. The product was recrystallized from ethylacetate (2.50 g, 5.52 mmol, 93% yield of white crystals). ^{1}H-NMR (300 MHz, CD_2Cl_2): δ 8.12 (dd, J = 8.5, 1.1 Hz, 1H, H_3), 7.58 (AB, J= 2.4, 1.1 Hz, 1H, H_6), 7.57 (AB, J= 2.4, 8.4 Hz, 1H, H_4), 7.36 (m, 2H, $H_{4',4''}$), 6.89 (m, 4H, $H_{3',5',3'',5''}$).

EXAMPLE 45

Preparation Of *N*-(2,6-Difluorobenzoyl)-5-Chloro-2-Nitroanilide

In this Example, *N*-(2,6-difluorobenzoyl)-5-chloro-2-nitroanilide (**700**) was prepared. First, *N,N*-bis-(2,6-difluorobenzoyl)-5-chloro-2-nitroanilide (**600**) (1.00 g, 2.20 mmol) was dissolved in methanol/dioxane (1:1) (20 mL), and then sodium hydroxide (92 mg, 2.30 mmol, 105 M%) was added. After stirring for 30 minutes at room temperature, additional sodium hydroxide (92 mg, 2.30 mmol, 105 M%) was added. After an additional 15 minutes the reaction was quenched with $NaHSO_4$,

diluted with CH_2Cl_2, washed with $NaHCO_{3\,(sat\ aq)}$ and $NaCl_{\,(sat\ aq)}$, dried (Na_2SO_4), filtered and concentrated. The product was recrystallized from ethylene oxide/hexane (3:1) to produce 0.55 g, (1.85 mmol, 84% yield) of white crystals. ^{1}H-NMR (200 MHz, CD_2Cl_2): δ 10.91 (s, 1H, NH), 9.04 (d, J= 2.3 Hz, 1H, H_6), 8.23 (d, J= 9.0 Hz, 1H, H_4), 7.52 (cm, 1H, $H_{4'}$), 7.26 (dd, J= 2.3, 9.0 Hz, 1H, H_4), 7.08 (m, 1H, $H_{3',5'}$).

EXAMPLE 46

Preparation Of *N*-(2,6-Difluorobenzoyl)-2-Amino-3-Nitroanilide

In this Example, *N*-(2,6-difluorobenzoyl)-2-amino-3-nitroanilide (**800**) was prepared according to Method A, described above, with the changes to the starting materials, notable variations, and/or additions to the method indicated as needed. Method A was also used to produce the compounds described in subsequent Examples, as indicated.

First, 3-nitro-1,2-phenylenediamine (13.3 g, 86.85 mmol) and 2,6-difluorobenzoyl chloride (**7**) (15.33 g, 10.95 mL, 86.85 mmol) were mixed, stirred overnight, and then recrystallized from ethylacetate/hexane to produce 12 g (41 mmol, 48% yield) of yellow crystals. ^{1}H-NMR (300 MHZ, DMSO-d_6): δ 10.23 (s, 1H, NH), 7.99 (dd, J= 8.7, 1.4 Hz, 1H, H_4), 7.71 (dd, J= 7.6 Hz, 1.4 Hz, 1H, H_6), 7.62 (m, 1H, $H_{4'}$), 7.28 (m, 2H, $H_{3',5'}$), 6.91 (s, 2H, NH_2), 6.76 (dd, J= 8.7, 7.6 Hz, 1H, H_5).

EXAMPLE 47

Preparation Of *N*-(2,6-Difluorobenzoyl)-2,3-Dimethyl-6-Nitroanilide

In this Example, *N*-(2,6-difluorobenzoyl)-2,3-dimethyl-6-nitroanilide (**900**) was prepared according to Method A, using 2,3-dimethyl-6-nitroaniline (2.00 g, 12.04 mmol) and 2,6-difluorobenzoyl chloride (**7**) (1.50 mL, 13.93 mmol, 115 M%). After 3 hours of mixing, a additional (**7**) (0.50 mL, 4.64 mmol, 40 M%) was added to the mixture. After an additional 5 hours of mixing, the compound was recrystallized from

ethylacetate/hexane (1:4) to produce 2.71 g (8.85 mmol, 74% yield) of yellow crystals. ^{1}H-NMR (300 MHz, CD_2Cl_2): δ 8.67 (s, 1H, NH), 7.81(d, J= 8.4 Hz, 1H, H_6), 7.48 (m, 1H, $H_{4'}$), 7.28 (d, J= 8.4 Hz, 1H, H_5), 7.05 (m, 2H, $H_{3',5'}$), 2.44 (s, 3H, CH_3), 2.31 (s, 3H, CH_3).

EXAMPLE 48

Preparation Of *N*-(2,6-Difluorobenzoyl)-3-Methyl-6-Nitroanilide

In this Example, *N*-(2,6-difluorobenzoyl)-3-methyl-6-nitroanilide (**1000**) was prepared according to Method A. First, 5-Methyl-2-nitroaniline (4.95 g, 32.5 mmol) was mixed with 2,6-difluorobenzoyl chloride (**7**) (4.50 mL, 41.8 mmol, 130 M%), as described. After recrystallization from ethylacetate, 8.71 g (29.8 mmol, 92 % yield) of yellow crystals were produced. ^{1}H-NMR (300 MHz, CD_2Cl_2): δ 10.85 (s, 1H, NH), 8.75 (s, 1H, H_2), 8.16 (d, J= 8.6, 1H, H_5), 7.50 (m, 1H, $H_{4'}$), 7.48 (d, J= 8.6, 1H, H_4), 7.06 (m, 2H, $H_{3'}$), 2.50 (s, 3H, CH_3).

EXAMPLE 49

Preparation Of *N*-(2,6-Difluorobenzoyl)-4-Methyl-2-Nitroanilide

In this Example, *N*-(2,6-difluorobenzoyl)-4-methyl-2-nitroanilide (**1100**) was prepared according to Method A. First, 4-Methyl-2-nitroaniline (4.95 g, 32.5 mmol) and was mixed with 2,6-difluorobenzoyl chloride (**7**) (4.50 mL, 41.8 mmol, 130 M%), as described. After recrystallization from ethylacetate, 6.69 g (22.9 mmol, 70 % yield) of yellow crystals were produced. ^{1}H-NMR (300 MHz, CD_2Cl_2): δ 10.64 (s, 1H, NH), 8.75 (d, J= 8.6, 1H, H_6), 8.06 (s, 1H, H_3), 7.52 (m, 1H, $H_{4'}$), 7.48 (d, J= 8.6, 1H, H_5), 7.06 (m, 2H, $H_{3',5'}$), 2.42 (s, 3H, CH_3).

EXAMPLE 50

Preparation Of *N*-(2,6-Difluorobenzoyl)-4-Bromo-2-Nitroanilide

In this Example, *N*-(2,6-difluorobenzoyl)-4-bromo-2-nitroanilide (**1200**) was prepared according to Method A. First, N-(2,6-difluorobenzoyl)-2-nitroanilide (1.20 g, 8.69 mmol) was suspended in 10 mL pyridine/THF (1:1). Bromine (0.5 mL) dissolved in acetic acid (0.5 mL) was then added to the mixture. After stirring for 1 hour at room temperature, the reaction was quenched with $NaHCO_{3\,(sat.\ aq)}$. The solution was extracted with CH_2Cl_2 and the organic extract was washed with $NaCl_{\,(sat.\ aq)}$, dried (Na_2SO_4), filtered and concentrated. The product was recrystallized from ethylacetate to produce 1.55 g, (4.34 mmol, 50 % yield) of yellow crystals. ^{1}H-NMR (200 MHz, CD_2Cl_2): δ 10.72 (br s, 1H, NH), 8.85 (d J= 9.1 Hz, 1H, H_5), 8.42 (d J= 2.4 Hz, 1H, H_3), 7.84 (ddd, J= 0.5, 2.4, 9.1 Hz, 1H, H_6), 7.52 (m, 1H, H_4), 7.07 (m, 2H, $H_{3',5'}$).

EXAMPLE 51

Preparation Of *N*-(2,6-Difluorobenzoyl)-
N-(2,6-Difluorobenzyl)-4-Bromo-2-Nitroanilide

In this Example, *N*-(2,6-difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-4-bromo-2-nitroanilide (**1400**) was prepared according to Method E. Method E was also used to produce the compounds described in subsequent Examples, with the changes to the starting materials, notable variations, and/or additions to the method indicated as needed.

N-(2,6-difluorobenzoyl)-4-bromo-2-nitroanilide (**1200**) (0.26 g, 0.73 mmol) and 2,6-difluoro-α-bromo-toluene (**1300**) (0.27 g, 1.30 mmol, 180 M%) were dissolved in THF (2 mL), to which NaH (0.15 g, 500 M%) was added. After 6 hours, the reaction was quenched with methanol and concentrated. The residue was redissolved in CH_2Cl_2, washed with $NaHCO_{3\,(sat.\ aq)}$ and $NaCl_{\,(sat.\ aq)}$, dried (Na_2SO_4), filtered and concentrated. The product was purified by flash chromatography eluting with

ethylacetate: hexane (1:4), and recrystallized from diethyl ether: hexane (3:1) to produce 0.26 g, (0.54 mmol, 74% yield) of white crystals. ^{1}H-NMR (200 MHz, DMSO d_6) (rotamers): δ 8.30 (d, J= 2.3 Hz, 1H, H_3, rotamer 1), 8.25 (d, J= 2.3 Hz, 1H, H_3, rotamer 2), 8.02 (dd, J= 2.3, 8.5 Hz, 1H, H_5, rotamer 1), 7.85 (dd, J= 2.3, 8.5 Hz, 1H, H_5, rotamer 2), 7.68 (m, 1H, $H_{4'}$, rotamer 1), 7.54-7.27 (m, 5H, $H_{6,4''}$ rotamers 1 & $H_{6,4',4''}$ rotamer 2), 7.16-6.94 (m, $H_{3',5',3'',5''}$ rotamers 1&2), 5.63 (d, J=14.3 Hz, 1H, CH_2PhF_2, rotamer 2), 4.99 (br, 1H, CH_2PhF_2, rotamer 1), 4.87 (br, 1H, CH_2PhF_2, rotamer 1), 4.86 (d, J=14.3 Hz, 1H, CH_2Ph, rotamer 2).

EXAMPLE 52

Preparation Of *N*-(2,6-Difluorobenzoyl)-*N*-(2,6-Difluorobenzyl)-4-Chloro-2-Nitroanilide

In this Example, *N*-(2,6-difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-4-chloro-2-nitroanilide (**1500**) was prepared according to Method E, using *N*-(2,6-difluorobenzoyl)-4-chloro-2-nitroanilide (**500**) (600 mg,1.92 mmol) mixed with 2,6-difluoro-α-bromo-toluene (**1300**) (478 mg, 2.3 mmol). After recrystallization from diethyl ether:hexane (3:1), 620 mg (1.41 mmol, 74%) of white crystals was produced. ^{1}H-NMR (300 MHz, CD_2Cl_2) (rotamers): δ 8.02 (d, J= 2.4 Hz, 1H, H_3, rotamer 1), 7.95 (d, J= 2.4 Hz, 1H, H_3, rotamer 2), 7.57 (dd, J= 8.6, 2.4 Hz, 1H, H_5, rotamer 1), 7.50 (m, 1H, $H_{4''}$, rotamer 1), 7.30 (dd, J= 8.4, 2.4 Hz, 1H, H_5, rotamer 2), 7.35-7.03 (m, 5H, $H_{4''}$ rotamer 2 & $H_{6,4'}$ rotamer 1&2), 6.94-6.68 (m, 8H, $H_{3',5',3'',5''}$, rotamer 1 & 2), 5.84 (d, J= 14.4, 1H, CH_2PhF_2, rotamer 2), 4.94 (br s, 2H, CH_2PhF_2, rotamer 1), 4.82 (d, J= 14.4 Hz, 1H, CH_2PhF_2, rotamer 2).

EXAMPLE 53

Preparation Of *N*-(2,6-Difluorobenzoyl)-
N-(2,6-Difluorobenzyl)-5-Chloro-2-Nitroanilide

In this Example, *N*-(2,6-difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-5-chloro-2-nitroanilide (**1600**) was prepared according to Method E. First, *N*-(2,6-difluorobenzoyl)-5-chloro-2-nitroanilide (**700**) (1.95 g, 6.24 mmol) and 2,6-difluoro-α-bromo-toluene (**1300**) (1.40 g, 6.76 mmol, 110 M%) were mixed for 2 hours. Then, an additional volume of (**1300**) (1.30 g, 100 M%) was added. After 5 hours of mixing, another additional volume of (**1300**) was added (0.75 g, 60 M%) was added . After mixing for 8 hours, purification by flash chromatography, elution with ethyl acetate: hexane (1:4) to produce 2.06 g (4.69 mmol, 75% yield) of white crystals. ^{1}H-NMR (200 MHz, DMSO d_6) (rotamers): δ 8.08 (d, J= 9.4 Hz, 1H, H_6, rotamer 1), 8.03 (d, J= 8.8 Hz, 1H, H_6, rotamer 2), 7.79-6.91 (m, 16H, $H_{3,4,3',4',5',3'',4'',5''}$ rotamer 1&2), 5.55 (d, J= 14.7, 1H, CH_2PhF_2, rotamer 2), 5.12 (br, 1H, CH_2PhF_2, rotamer 1), 4.99 (d, J= 14.7 Hz, 1H, CH_2PhF_2, rotamer 2), 4.87 (br, 1H, CH_2PhF_2, rotamer 1).

EXAMPLE 54

Preparation Of *N*-(2,6-Difluorobenzoyl)-
N-(2,6-Difluorobenzyl)-4-Methyl-2-Nitroanilide

In this Example, *N*-(2,6-difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-4-methyl-2-nitroanilide (**1700**) was prepared according to Method E. *N*-(2,6-difluorobenzoyl)-4-methyl-2-nitroanilide (**1100**)(2.00 g, 6.84 mmol) and 2,6-difluoro-α-bromo-toluene (**1300**) (2.12 g, 10.2 mmol, 150 M%) were mixed for 3 hours, and recrystallized from diethyl ether: hexane (3:1) to produce 2.80 g (6.69 mmol, 98% yield) of white crystals. ^{1}H-NMR (300 MHz, DMSO-d_6) (rotamers): δ 7.89 (dd, J= 2.0, 0.9 Hz, 1H, H_3 rotamer 1), 7.87 (dd, J= 2.0, 0.8 Hz, 1H, H_3 rotamer 2), 7.66 (m, 1H, H_4 rotamer 1), 7.56 (ddd, J= 8.2, 2.0, 0.9 Hz, 1H, H_5 rotamer 1), 7.43 (m, 1H, H_4 rotamer 2),

7.37-7.27 (m, 5H, H_5 rotamer 2, $H_{3',5'}$ rotamer 1, $H_{4''}$ rotamer 1&2) 7.21 (d, J=8.2 Hz, 1H, H_6 rotamer 1), 7.07 (m, 2H, $H_{3',5'}$ rotamer 2), 7.01-6.90 (m, 4H, $H_{3'',5''}$ rotamer 1&2), 6.83 (br d, J= 7.7 Hz, 1H, H_6 rotamer 2), 5.71 (d, J= 14.4 Hz, 1H, $CHPhF_2$ rotamer 2), 4.97 (d, J= 14.4 Hz, 1H, $CHPhF_2$ rotamer 1), 4.83 (d, J= 14.4 Hz, 1H, $CHPhF_2$ rotamer 1), 4.78 (d, J= 14.4 Hz, 1H, $CHPhF_2$ rotamer 2), 2.41 (s, 3H, CH_3 rotamer 1), 2.27 (s, 3H, CH_3 rotamer 2).

EXAMPLE 55

Preparation Of *N*-(2,6-Difluorobenzoyl)-
N-(2,6-Difluorobenzyl)-5-Methyl-2-Nitroanilide

In this Example, *N*-(2,6-difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-5-methyl-2-nitroanilide (**1800**) was prepared according to Method E. *N*-(2,6-difluorobenzoyl)-3-methyl-6-nitroanilide (**1000**) (2.00 g, 6.84 mmol) and 2,6-difluoro-α-bromo-toluene (**1300**) (2.12 g, 10.2 mmol, 150 M%) were mixed for 3 hours. After recrystallization from diethyl ether: hexane (3:1) 1.92 g (4.59 mmol, 67% yield) of white crystals were produced. ^{1}H-NMR (200 MHz, CD_3OD) (rotamers): δ 7.92 (d, J= 8.5 Hz, 1H, H_6, rotamer 1), 7.89 (d, J= 8.5, 1H, H_6, rotamer 2), 7.60 (m, 1H, $H_{4'}$, rotamer 1), 7.44-6.72 (m, 15H, $H_{3,4,3',5',3'',4'',5''}$ rotamers 1 & $H_{3,4,3',4',5',3'',4'',5''}$ rotamer 2), 5.86 (d, J= 14.3 Hz, 1H, CH_2PhF_2, rotamer 2), 4.98 (br, 2H, CH_2PhF_2, rotamer 1), 4.88 (d, J= 14.3 Hz, 1H, CH_2PhF_2, rotamer 2), 2.35 (s, 3H, CH_3, rotamer 1), 2.35 (s, 3H, CH_3, rotamer 2).

EXAMPLE 56

Preparation Of *N*-(2,6-Difluorobenzoyl)-
N-(2,6-Difluorobenzyl)-2-Methyl-6-Nitroanilide

In this Example, *N*-(2,6-difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-2-methyl-6-nitroanilide (**1900**) was prepared according to Method E. First, 2,6-difluorobenzoyl-2-methyl-6-nitroanilide (11) (450 mg, 1.54 mmol) and 2,6-difluoro-α-bromo-toluene

(**1300**) (351 mg, 1.69 mmol) were mixed. After recrystallization from diethylether/methanol 490 mg (1.17 mmol, 76% yield) of colorless crystals was produced. ^{1}H-NMR (300 MHZ, CD_2C1_2): δ 7.82 (dd, J= 8.0, 1.5 Hz, 1H, H_5), 7.52 (m, 1H, H_{4bz}), 7.51 (dd, 1H, J= 7.9, 1.6 Hz, 1H, H_3), 7.42 (t, 1H, J= 7.9 Hz, H_4), 7.25 (m, 1H, H_{4bn}), 7.12 (m, 2H, $H_{3bz,5bz}$), 6.74 (m, 2H, $H_{3bn,5bn}$), 4.80 (s, 2H, CH_2), 2.17 (s, 3H, CH_3).

EXAMPLE 57

Preparation Of 1-(2,6-Difluorobenzyl)-
2-(2,6-Difluorophenyl)-5-Bromobenzimidazole

In this Example, 1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-5-bromobenzimidazole (**2100**) was prepared according to Method F. Method F was also used to produce the compounds described in subsequent Examples, with the changes to the starting materials, notable variations, and/or additions to the method indicated as needed.

First, 2,6-difluoro-α-bromo-toluene (**1300**) (0.26 g, 0.54 mmol) was dissolved in glacial acetic acid (5 mL). Then, iron powder (**17**) (0.55 g) was added to the mixture. After 30 min, the reaction was concentrated to dryness, diluted with ethyl acetate, and adjusted to pH 7 with $NaHCO_{3\,(sat\ aq)}$. The organic solution was collected and washed with $NaHCO_3$ and NaCl, dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 2% methanol/CH_2Cl_2 and then recrystallized from 3:1 : hexane to produce 0.14 g (0.33 mmol, 62% yield) of white crystals. ^{1}H-NMR (300 MHz, CD_2Cl_2): δ 8.11 (dd, J= 0.6, 1.9 Hz, 1H, H_4), 7.56 (cm, 1H, $H_{4'}$), 7.41 (AB, J= 1.9, 8.7 Hz, 1H, H_5), 7.40 (AB, J= 0.6, 8.7 Hz, 1H, H_6), 7.26 (cm, 1H, $H_{4'}$), 7.10 (cm, 2H, $H_{3',5'}$), 6.83 (cm, 2H, $H_{3',5'}$), 5.35 (s, 2H, CH_2PhF_2). Anal. ($C_{20}H_{11}BrF_4N_2$ x3/4H_2O) C,H,N.

EXAMPLE 58

Preparation Of 1-(2,6-Difluorobenzyl)-
2-(2,6-Difluorophenyl)-5-Chlorobenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-5-chlorobenzimidazole (**2200**) was prepared according to Method F. *N*-(2,6-difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-4-chloro-2-nitroanilide (**1500**) (620 mg, 1.41 mmol) and iron powder (**17**) (200 mg) were mixed for 3 hours. After recrystallization, 250 mg (0.64 mmol, 45%) of colorless crystals were produced, with a mp of 136°C. ^{1}H-NMR (300 MHZ, CD_2C1_2): δ 7.77 (d, J= 1.95 Hz, H_4), 7.55 (m, 1H, $H_{4'}$), 7.42 (d, J= 8.7 Hz, 1H, H_7), 7.27 (dd, J= 8.7, 1.95 Hz, 1H, H_6), 7.26 (m, 1H, $H_{4''}$), 7.09 (m, 2H, $H_{3',5'}$), 6.82 (m, 2H, $H_{3'',5''}$), 5.34 (s, 2H, CH_2). Anal. ($C_{20}H_{11}ClF_4N_2$) C,H,N.

EXAMPLE 59

Preparation Of 1-(2,6-Difluorobenzyl)-
2-(2,6-Difluorophenyl)-6-Chlorobenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-6-chlorobenzimidazole (**2300**) was prepared according to Method F. *N*-(2,6-difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-5-chloro-2-nitroanilide (**1600**) (0.57 g, 1.30 mmol) and iron powder (**17**) (0.43 g) were mixed for 1 hour, and purified by flash chromatography eluted with 2% methanol/CH_2Cl_2, and recrystallized from diethyl ether: hexane (3:1), to produce 0.43 g (1.10 mmol, 85% yield) of white crystals. ^{1}H-NMR (300 MHz, CD_2Cl_2): δ 7.73 (dd, J= 0.9, 8.6 Hz, 1H, H_4), 7.56 (m, 1H, $H_{4''}$), 7.51 (d, J= 1.9 Hz, 1H, H_7), 7.29 (dd, J= 1.9, 8.6 Hz, 1H, H_5), 7.27 (m, 1H, $H_{4'}$), 7.09 (m, 2H, $H_{3'',5''}$), 6.84 (m, 2H, $H_{3',5'}$), 5.33 (s, 2H, CH_2PhF_2). Anal. ($C_{20}H_{11}ClF_4N_2$) C,H,N.

EXAMPLE 60

Preparation Of 1-(2,6-Difluorobenzyl)-
2-(2,6-Difluorophenyl)-5-Methylbenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-5-methylbenzimidazole (**2400**) was prepared according to Method F. Iron powder (**17**) (1.55 g, 3.71 mmol) and iron powder (**17**) (0.79 g) were mixed, and then recrystallized from diethyl ether: hexane (3:1), to produce 0.85 g (2.30 mmol, 62% yield) of white crystals. ^{1}H-NMR (300 MHz, CD_2Cl_2): δ 7.60-7.49 (cm, 2H, $H_{4,4''}$), 7.37 (d, J= 8.4 Hz, 1H, H_7), 7.24 (m, 2H, $H_{4'}$), 7.14 (m, 1H, H_6), 7.09 (m, 2H, $H_{3'',5''}$), 6.81 (m, 2H, $H_{3',5'}$), 5.34 (s, 2H, CH_2PhF_2), 2.47 (s, 3H, CH_3). Anal. ($C_{21}H_{14}F_4N_2$) C,H,N.

EXAMPLE 61

Preparation Of 1-(2,6-Difluorobenzyl)-
2-(2,6-Difluorophenyl)-6-Methylbenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-6-methylbenzimidazole (**2500**) was prepared according to Method F. *N*-(2,6-difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-5-methyl-2-nitroanilide (**1800**)(1.56 g, 3.73 mmol) and iron powder (**17**) (0.79 g) were mixed. After recrystallization from diethyl ether: hexane 0.89 g (2.41 mmol, 64% yield) of white crystals were produced. ^{1}H-NMR (300 MHz, CD_2Cl_2): δ 7.64 (cm, 1H, H_4), 7.52 (m, 1H, $H_{4''}$), 7.30-7.19 (m, 2H, $H_{7,4'}$), 7.14-7.03 (m, 3H, $H_{5,3'',5''}$), 6.81 (m, 2H, $H_{3',5'}$), 5.33 (s, 2H, CH_2PhF_2), 2.48 (s, 3H, CH_3). Anal. ($C_{21}H_{14}F_4N_2$) C,H,N.

EXAMPLE 62

Preparation Of 1-(2,6-Difluorobenzyl)-
2-(2,6-Difluorophenyl)-7-Methylbenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-7-methylbenzimidazole (**2600**) was prepared according to Method F. *N*-(2,6-difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-2-methyl-6-nitroanilide (**1900**) (300 mg, 0.72 mmol) and (**20**) (50 mg) were mixed. After recrystallization from ethyl acetate, 149 mg (0.15 mmol, 56% yield) of colorless crystals were produced, with a mp of 177°C. ^{1}H-NMR (300 MHz, CD_2Cl_2): 7.62 (d, J= 8.2 Hz, 1H, H_4), 7.45 (m, 1H, $H_{4'}$), 7.18 (m, 1H, $H_{4''}$), 7.17 (dd, J= 7.3, 8.2, 1H, H_5), 7.08 (d, J= 7.3, H_6), 6.95 (m, 2H, $H_{3',5'}$), 6.70 (m, 2H, $H_{3'',5''}$), 5.64 (s, 2H, CH_2), 2.74 (s, 3H, CH_3). Anal. ($C_{21}H_{14}F_4N_2$) C,H,N.

EXAMPLE 63

Preparation Of 2-(2,6-Difluorophenyl)-4,5-Dimethylbenzimidazole

In this Example, 2-(2,6-difluorophenyl)-4,5-dimethylbenzimidazole (**2700**) was produced according to Method F. *N*-(2,6-difluorobenzoyl)-2,3-dimethyl-6-nitroanilide (**900**) (1.40 g, 4.57 mmol) and (**20**) (1.05 g) were mixed for 1 h, and recrystallized from ethylacetate to produce 1.07 g (4.14 mmol, 91% yield) of white crystals. ^{1}H-NMR (300 MHz, CD_2Cl_2): δ 7.49-7.37 (m, 2H, $H_{4',7}$), 7.16-7.07 (m, 3H, $H_{3',5',6}$), 2.54 (br s, 3H, CH_3), 2.42 (s, 3H, CH_3).

EXAMPLE 64

Preparation Of 2-(2,6-Difluorophenyl)-4-Nitrobenzimidazole

In this Example, 2-(2,6-difluorophenyl)-4-nitrobenzimidazole (**2800**), according to Method F. *N*-(2,6-difluorobenzoyl)-2-amino-3-nitroanilide (**800**)(12 g, 41 mmol) was dissolved in 130 mL of acetic acid, heated to reflux, and stirred for 12 hours. The reaction mixture was cooled to room temperature, neutralized with NaOH (4N),

basified with $NaHCO_3$ (1%), and extracted with ethylacetate (3 x 300 mL). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated. The remaining crystals were recrystallized from ethylacetate/hexane to produce 7.7 g, (28 mmol, 68%) of crystals. ^{1}H-NMR (300 MHZ, CD_2C1_2): δ 11.00 (s, 1H, NH), 8.23 (dd, J= 8.2, 0.8 Hz, 1H, H_5), 8.21 (dd, J= 8.0, 0.8 Hz, 1H, H_7), 7.54 (m, 1H, $H_{4'}$), 7.45 (dd, J= 8.0, 8.2 Hz, 1H, H_6), 7.13 (m, 2H, $H_{3',5'}$).

EXAMPLE 65

Preparation Of 2-(2,6-Difluorophenyl)-5-Nitrobenzimidazole

In this Example, 2-(2,6-difluorophenyl)-5-nitrobenzimidazole (**2900**) was prepared according to Method F. 2-(2,6-difluorophenyl)-benzimidazole(**11**) (2.00 g, 8.70 mmol) was dissolved in H_2SO_4 (5.0 mL), and HNO_3 (5.0 mL) was added. After 2 hours at room temperature, the reaction was quenched with ice (50 mL), filtered and washed with water yielding a white solid (1.92 g, 80% yield). ^{1}H-NMR (300 MHz, CD_2Cl_2): δ 8.60 (d, J= 2.2 Hz, 1H, H_4), 8.25 (dd, J= 2.2, 8.9 Hz, 1H, H_7), 7.78 (d, J= 8.9 Hz, 1H, H_6), 7.65 (m, 1H, $H_{4'}$), 7.24 (m, 2H, $H_{3',5'}$).

EXAMPLE 66

Preparation Of 1-(2,6-Difluorobenzyl)-
2-(2,6-Difluorophenyl)-4,5-Dimethylbenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-4,5-dimethylbenzimidazole (**3000**) was prepared according to Method E. 2-(2,6-difluorophenyl)-4,5-dimethylbenzimidazole (**2700**)(0.25 g, 0.97 mmol) and 2,6-difluoro-α-bromo-toluene (**1300**) (0.44 g, 2.12 mmol, 220 M%) were mixed. After flash chromatography, elution with 4% MeOH/CH_2Cl_2, and recrystallization from ethylacetate/hexane (1:1) 0.38 g (0.81 mmol, 83% yield) of white crystals was produced. ^{1}H-NMR (300 MHz, CD_3OD): δ 7.63 (cm, 1H, $H_{4'}$), 7.34 (cm, 1H, H_6), 7.30 (cm, 1H, $H_{4''}$), 7.16 (cm, 1H, H_7), 7.13 (cm, 2H, $H_{3',5'}$), 6.85 (cm, 2H, $H_{3'',5''}$),

5.40 (s, 2H, CH_2PhF_2), 2.54 (s, 3H, CH_3), 2.40 (s, 3H, CH_3). Anal. ($C_{22}H_{16}F_4N_2$) C,H,N.

EXAMPLE 67

Preparation Of 1-(2,6-Difluorobenzyl)-
2-(2,6-Difluorophenyl)-4-Nitrobenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-4-nitrobenzimidazole (**3100**) was prepared according to Method E. 2-(2,6-difluorophenyl)-4-nitrobenzimidazole (**2800**) (7.7 g, 28 mmol) and 2,6-difluoro-α-bromo-toluene (**1300**) (6.95 g, 33.6 mmol) were mixed as described. After recrystallization from diethyl ether:hexane (3:1) 9.8 g (24.4 mmol, 87%) of slightly brown crystals were produced, with a mp of 169°C. ^{1}H-NMR (300 MHZ, CD_2C1_2): δ 8.13 (dd, J= 8.1, 0.92 Hz, 1H, H_5), 7.86 (dd, J= 8.1, 0.9 Hz, 1H, H_7), 7.59 (m, 1H, $H_{4'}$), 7.43 (dd, J= 8.1 Hz, H_6), 7.28 (m, 1H, $H_{4''}$), 7.12 (m, 2H, $H_{3',5'}$), 6.84 (m, 2H, $H_{3'',5''}$), 5.44 (s, 2H, CH_2). Anal. ($C_{20}H_{11}F_4N_3O_2$) C,H,N.

EXAMPLE 68

Preparation Of 1-(2,6-Difluorobenzyl)-
2-(2,6-Difluorophenyl)-5-Nitrobenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-5-nitrobenzimidazole (**3200**) was prepared according to Method E. 2-(2,6-difluorophenyl)-5-nitrobenzimidazole (**2900**) (0.91 g, 3.31 mmol) and 2,6-difluoro-α-bromo-toluene (**1300**) (1.08 g, 5.22 mmol, 160 M%) were mixed, and a second addition of (**1300**) (0.47 g, 2.27 mmol, 70 M%) was added to the mixture after 1 hour of mixing. After flash chromatography, elution with ethyl acetate:hexane (1:4) 1.09 g (2.71 mmol, 82% yield) of white crystals were produced. ^{1}H-NMR (300 MHz, CD_2Cl_2): δ 8.69 (dd, J= 0.5, 2.2 Hz, 1H, H_4), 8.23 (dd, J= 2.2, 9.0 Hz, 1H, H_6), 7.59

(dd, J= 0.5, 9.0 Hz, 1H, H_7), 7.59 (m, 1H, $H_{4'}$), 7.28 (m, 1H, $H_{4''}$), 7.12 (m, 2H, $H_{3',5'}$), 6.84 (m, 2H, $H_{3'',5''}$), 5.44 (s, 2H, CH_2PhF_2). Anal. ($C_{20}H_{11}F_4N_3O_2$) C,H,N.

EXAMPLE 69

Preparation Of 4-Amino-1-(2,6-Difluorobenzyl)-
2-(2,6-Difluorophenyl)Benzimidazole

In this Example, 4-amino-1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole (**3300**) was produced according to Method E. 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-4-nitrobenzimidazole (**3100**)(9.2 g, 23 mmol) was dissolved in acetic acid (130 mL), and $SnCl_2x2H_2O$ (41.5 g) dissolved in $HCl_{(con)}$ (35 mL) was added. After stirring for 3 hours at room temperature, the mixture was neutralized with NaOH (4N), basified with $NaHCO_3$ (5%), diluted with water to give a final volume of 3 L, and then extracted with ethylacetate (5 x 300 mL). The combined ethylacetate layers were dried (Na_2SO_4), filtered, and evaporated. The residue was purified by gravity chromatography, eluting with acetone/hexane (1:1) and recrystallized from acetone/hexane/diethylether to produce 3.7 g of pink crystals (10 mmol, 44% yield), with a mp of 178°C. ^{1}H-NMR (300 MHZ, CD_2C1_2): δ 7.52 (m, 1H, $H_{4'}$), 7.23 (m, 1H, $H_{4''}$), 7.07 (m, 2H, $H_{3',5'}$), 7.06 (dd, J= 8.1, 7.7 Hz, 1H, H_6), 6.82 (d, J= 8.1 Hz, H_7), 6.81 (m, 2H, $H_{3'',5''}$), 6.52 (d, J= 7.7, 0.9 Hz, 1H, H_5), 5.29 (s, 2H, CH_2), 4.42 (s, 2H, NH_2). Anal. ($C_{20}H_{13}F_4N_3$) C,H,N.

EXAMPLE 70

Preparation Of 4-Bromo-1-(2,6-Difluorobenzyl)-
2-(2,6-Difluorophenyl)Benzimidazole

In this Example, 4-bromo-1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole (**3400**) was produced according to Method E. 4-amino-1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole (**3300**)(800 mg, 2.15 mmol) was suspended in HBr (48%, 7 mL) at 0°C, then $NaNO_2$ (193 mg, 2.8 mmol)

in water (1.5 mL) was slowly added. After stirring for 30 min at 0-5°C, the mixture was added to CuBr (373 mg, 2.6 mmol) dissolved in HBr (48%, 3 mL). After 30 min at room temperature, water (250 mL) was added, and the pH adjusted to 5 with KOH 4N. The mixture was extracted with ethylacetate, dried (Na_2SO_4), filtered, and evaporated. The crude brown crystals were purified by gravity chromatography eluting with hexane/acetone (2:1) and recrystallized with diethylether/hexane (3:1) to produce 610 mg, (1.4 mmol, 65% yield) of colorless crystals, with a mp of 147°C. ^{1}H-NMR (300 MHZ, CD_2C1_2): δ 7.56 (m, 1H, H_4·), 7.49 (dd, J= 7.7, 0.87 Hz, 1H, H_5), 7.47 (d, J= 7.9 Hz, H_7), 7.26 (m, 1H, $H_{4''}$), 7.18 (dd, J= 8.2, 7.7 Hz, 1H, H_6), 7.09 (m, 2H, $H_{3',5'}$), 6.82 (m, 2H, $H_{3'',5''}$), 5.35 (s, 2H, CH_2). Anal. ($C_{20}H_{11}BrF_4N_2$ x1/4H_2O) C,H,N.

EXAMPLE 71

Preparation Of 4-Chloro-1-(2,6-Difluorobenzyl)-2-(2,6-Difluorophenyl)Benzimidazole

In this Example, 4-chloro-1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole (**3500**) was prepared according to Method E. 4-amino-1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole (**3300**) (800 mg, 2.15 mmol) was dissolved in $HC1_{(con)}$ (7 mL) and water (5 mL) at 0°C, then $NaNO_2$ (193 mg, 2.8 mmol) in water (1.5 mL) was slowly added. After 30 min at 0-5°C, the mixture was added to CuC1 (256 mg, 2.6 mmol) in HCl_{con} (2 mL) at 0°C. After rising to room temperature over 40 min, the pH was adjusted to pH 5, diluted with water (80 mL), extracted with ethylacetate, dried (Na_2SO_4), filtered, and evaporated. The residue was purified by gravity chromatography eluting with hexane/acetone (2:1) and recrystallized from acetone/hexane to produce 350 mg of yellow crystals (0.90 mmol, 42% yield), with a mp of 163°C. ^{1}H-NMR (300 MHZ, CD_2C1_2): δ 7.56 (m, 1H, H_4·), 7.43 (dd, J= 8.0, 1.1 Hz, 1H, H_7), 7.31 (dd, J=7.8, 1.1 Hz, 1H, H_5), 7.26 (m, 1H, $H_{4''}$), 7.24 (dd, J= 8.0, 7.8 Hz 1H, H_6), 7.09 (m, 2H, $H_{3',5'}$), 6.83 (m, 2H, $H_{3'',5''}$), 5.36 (s, 2H, CH_2). Anal. ($C_{20}H_{11}ClF_4N_2$) C,H,N.

EXAMPLE 72

Preparation Of 1-(2,6-Difluorobenzyl)-
2-(2,6-Difluorophenyl)-4-Acetamidobenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-4-acetamidobenzimidazole (**3600**) was prepared according to Method E. 4-amino-1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole (**3300**)(0.30 g, 0.81 mmol) was dissolved in THF (3 mL), and acetic anhydride (100 mL, 1.06 mmol) was then added. After 3 hours, additional acetic anhydride (20 mL, 0.21 mmol) was added. After 5 hours, the reaction was concentrated to dryness, diluted with ethyl acetate (50 mL), washed with $NaHCO_3$ (25 mL) and NaCl (25 mL), dried (Na_2SO_4), filtered, and concentrated. The product was recrystallized from diethyl ether:hexane (3:1) to produce 0.32 g (0.77 mmol, 95% yield) of white crystals. ^{1}H-NMR (300 MHz, CD_2Cl_2): δ 8.49 (1H, br, NH), 8.20 (d, J= 7.8 Hz, 1H, H_5), 7.56 (m, 1H, $H_{4'}$), 7.26 (t, J= 7.9 Hz, 1H, H_6), 7.26 (m, 1H, $H_{4''}$), 7.19 (d, J= 7.9 Hz, 1H, H_7), 7.10 (m, 2H, $H_{3',5'}$), 6.82 (m, 2H, $H_{3'',5''}$), 5.34 (s, 2H, CH_2PhF_2), 2.21 (s, 3H, Ac). Anal. ($C_{22}H_{15}F_4N_3O$) C,H,N.

EXAMPLE 73

Preparation Of 1-(2,6-Difluorobenzyl)-
2-(2,6-Difluorophenyl)-4-*N*,*N*-Dimethylaminobenzimidazole

In this Example, 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-4-*N*,*N*-dimethylaminobenzimidazole (**3700**) was prepared according to Method E. A slurry of 4-amino-1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole (**3300**)(0.37 g, 1.0 mmol) and sodium borohydride (0.27 g) was added to a mixture of 3 M H_2SO_4 (0.80 mL) and 37% H_2CO (0.50 mL). After the addition was complete, the mixture was concentrated to dryness, diluted with ethylene acetate, washed with Na_2CO_3 and NaCl, dried (Na_2SO_4), filtered, concentrated and recrystallized from diethyl ether:hexane (3:1) to produce 0.34 g (0.85 mmol, 85% yield) of white crystals. ^{1}H-NMR (300 MHz,

CD_2Cl_2): δ 7.51 (m, 1H, $H_{4'}$), 7.23 (m, 1H, $H_{4''}$), 7.13 (t , J= 8.0 Hz, 1H, H_6), 7.06 (m, 2H, $H_{3',5'}$), 6.90 (d, J= 8.0 Hz, 1H, H_5), 6.80 (m, 2H, $H_{3'',5''}$), 6.48 (d, J= 8.0 Hz, 1H, H_7), 5.30 (s, 2H, CH_2PhF_2), 3.18 (s, 6H, $N(CH_3)_2$). Anal. ($C_{22}H_{17}F_4N_3$) C,H,N.

EXAMPLE 74

Reverse Transcriptase Assay

In this Example, the effects of various compounds were tested for their ability to inhibit the RNA-dependent DNA polymerase (*i.e.*, RT) activity of purified RT. Briefly, in the basic assay (*e.g.*, controls), purified RT protein (0.015 mg/mL) was incubated in a 100 μL reaction mixture containing 25 mM Tris (pH 8.0), 75 mM KCl, 8 mM $MgCl_2$, 2 mM dithiothreitol, 0.1 units poly (rC)-oligo(dG), 0.01 mM dGTP, 1x BSA, 10 mM CHAPS, 0.025 mCi (α ^{35}S)dGTP (specific activity, 1000 Ci/mmol), for 1 hour at 37°C. In the test assays, various concentrations of anti-RT compounds were included in the reaction mixture. The assays were stopped by adding 1 mL of 10% trichloroacetic acid and 30 μL of denatured and sheared salmon sperm DNA (10 mg/mL) as a carrier. The labeled polymer was collected on Whatman glass GF/C filters by suction filtration, washed with 10% trichloroacetic acid and 95% ethanol, and the radioactivity was counted.

Figure 7 shows the structures, formulae, weight, and the percentage of various compounds produced using the methods of the present invention, as well as remaining HIV RT activity as reported as a percent of the control in HIV RT inhibition assays.

EXAMPLE 75

Cytopathic Cell Killing Anti-Viral Assay

In this Example, the antiviral and cellular toxicity of NNRTIs was investigated, using the cytopathic cell killing assay described by Yang (Yang *et al.*, "Characteristics of a Group of Nonnucleoside Reverse Transcriptase Inhibitors with Structural Diversity and Potent Anti-Human Immunodeficiency Virus Activity," Leukemia 9:S75-S85

[1995]). Briefly, in this method cells (*e.g.*, the CEM-SS cell line, available from the NIAID AIDS Research and Reference Program [ARRRP]) were seeded at a density of 5×10^3 cells/well, into the wells of a 96-well microtiter plate. The cells were then infected with HIV virus (either mutant or WT), at a multiplicity of infection (MOI) previously determined to provide complete cell killing by 6 days of culture post-infection (*e.g.*, MOI of 0.01-0.05). Each of the HIV isolates was pre-titered to induce equivalent levels of infection based on cell killing or virus production, prior to their use in these assays. A range of test compound concentrations was added to the wells in triplicate (*e.g.*, serial half-log dilutions) to evaluated inhibition of HIV infection. Controls for each assay included drug controls (drug colorimetric control wells), drug cytotoxicity control wells (cells with drug), virus control wells (cells with virus), and cell viability controls (cells only). Positive control drugs (*e.g.*, AZT and ddC) were run in parallel as positive control drugs.

After six days of incubation at 37°C, cell viability was determined spectrophotometrically at 450 nm for each well, using the metabolic reduction of XTT to a soluble colored formazan. (*See*, Gartner and Popovic, "Virus Isolation and Production, *in* Aldovini and Walker (eds.), *Techniques in HIV Research*, Stockton Press, NY, pp. 69-63 [1991]; and Nara and Fischinger, "Quantitative Infectivity Assay for HIV-1 and HIV-2," Nature 332:469-470 [1988]).

Antiviral and toxicity data were reported as the quantity of drug required to inhibit 50% of virus-induced cell killing or virus production (EC_{50}), and the quantity of drug required to reduce cell viability by 50% (IC_{50}). The *in vitro* therapeutic index (TI_{50}) was defined as the fold-difference between the EC_{50} and IC_{50}. The results for the various compounds are included in Figures 8 and 19. In addition, graphs showing the summary data for three compounds are shown in Figures 20-22. Figure 20 shows the graph for compound **33**. As indicated in this Figure, **33** exhibited very effective therapeutic dose. Figure 21 shows the graph for compound **34**, another compound that also exhibited an effective therapeutic dose. Figure 22 shows the graph for compound **2100**, a compound that was found to be inactive.

EXAMPLE 76

Production Of Inactive Compounds Compared With Active Compounds

In this Example, an intermediate (1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-4-aminobenzimidazole) used in a previous synthesis to prepare 1-2(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole was brominated to investigate the effects of this process on the compound. The product formed was found to be ineffective in inhibiting HIV RT. This and four other compounds synthesized during the development of the present invention, but found to be unsuitable for using as inhibitors of HIV RT are shown in Figure 9.

Based on these results and comparisons with previously described, substitutions of the benzimidazole core ring that comprised H, CH_3, Cl, Br, and $N(CH_3)_2$ were found to inhibit HIV RT, while substitutions with NO_2, and NHAc did not inhibit the enzyme. These substitutions are illustrated in Figure . Although it is not necessary for an understanding of the present invention, it was determined that electron donating or halogen groups apparently increase RT inhibition activity, while electron withdrawing groups decrease inhibition activity. Furthermore, it is apparent that only the 4,6 positions can be substituted, as substitutions by any groups at the 5 or 7 positions consistently resulted in reduced to no RT inhibition activity. In addition, it was observed that substitutions of the 4 position with electron donating or halogen groups led to greater HIV RT inhibition than substitution at the 6 position.

From the above, it is clear that the present invention provide compositions and methods for the treatment of HIV infection. In particular, the present invention provides non-nucleoside inhibitors of reverse transcriptase (RT). In particular, the present invention relates to stable analogues of 1-(2,6-difluorophenyl)-1H,3H-thiazolo[3,4-a]benzimidazole, effective in the inhibition of human immunodeficiency virus (HIV) RT, with particular activity against HIV-1 RT. Furthermore, the present invention provides highly purified compositions with high activity against HIV-1 RT

mutants that are refractory to inhibition with other non-nucleoside HIV-1 RT compounds.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

CLAIMS

1. A 1-aryl-2-(2,6-difluorophenyl)-benzimidazole composition with general structure of Figure 12, wherein X is selected from the group consisting of H and methyl, and wherein R'' is selected from the group consisting of 2,6-difluorobenzyl, benzyl, 2,6-dichlorobenzyl, 2,3,4,5,6-pentafluorobenzyl, pyridylmethyl, benzenesulfonyl, and 2,6-difluorophenylbenzoyl.

2. A 1-(2,6-difluorophenyl)-2-benzimidazole composition with the general structure of Figure 13, wherein X' is selected from the group consisting of H and methyl, and wherein R' is selected from the group consisting of phenyl, formyl, isopropyl, H, methyl, hydroxymethyl, difluorobenzyloxymethyl, 2,6 diflourophenyl, methylphenyl, pyridyl, and naphthyl.

3. A 4,5,6, or 7-substituted-1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole composition of the general structure of Figure 23, wherein X'''' is selected from the group consisting of methyl, 4-methyl, 5-methyl, 6-methyl, 7-methyl, and 4,5-methyl, 4-chloro, 5-chloro, 6-chloro, 4-bromo, 5-bromo, 4-nitro, and 5-nitro.

4. A 4-substituted-1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole composition of the general structure of Figure 24, wherein X''' is selected from the group consisting of methyl, amine, acetamide, dimethylamine, bromine, and chlorine.

5. A method for treatment of human immunodeficiency virus infection, comprising the steps of:

a) providing: i) a subject suspected of being infected with human immunodeficiency virus; and ii) a composition having anti-reverse transcriptase activity, wherein said composition comprises at least one substituted benzimidazole, wherein said substituted benzimidazole contains at least one substitution at the C-2 site, and at least one substitution at the N-1 site;

b) exposing said subject to said composition; and

c) observing for inhibition of said anti-reverse transcriptase activity.

6. The method of Claim 5, wherein said human immunodeficiency virus is HIV-1.

7. The method of Claim 5, wherein said substituted benzimidazole is of the general structure of Figure 12.

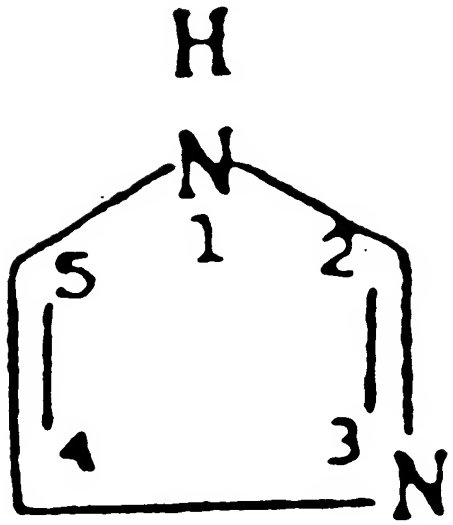
8. The method of Claim 5, wherein said substituted benzimidazole is of the general structure of Figure 13.

9. The method of Claim 5, wherein said substituted benzimidazole is of the general structure of Figure 23.

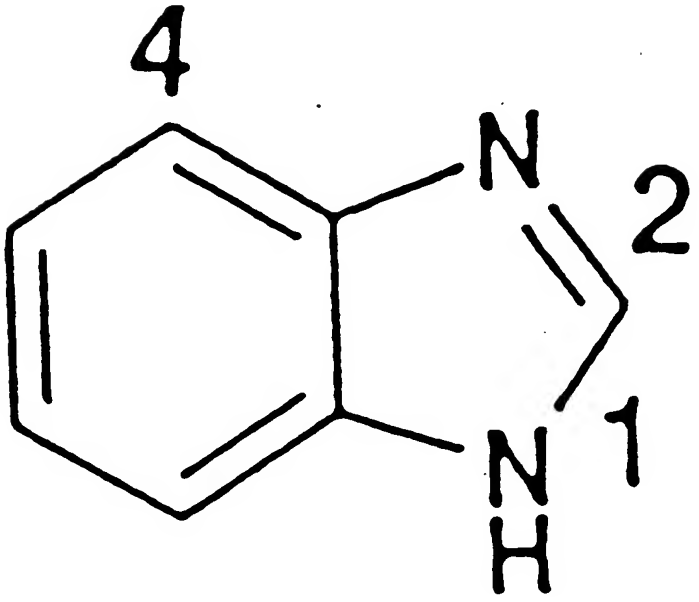
ABSTRACT

The present invention provides compositions and methods for the treatment of HIV infection. In particular, the present invention provides non-nucleoside inhibitors of reverse transcriptase (RT). The present invention is related to non-nucleoside inhibitors of reverse transcriptase (RT). In particular, the present invention relates to a novel class of substituted benzimidazoles, effective in the inhibition of human immunodeficiency virus (HIV) RT.

FIGURE 1

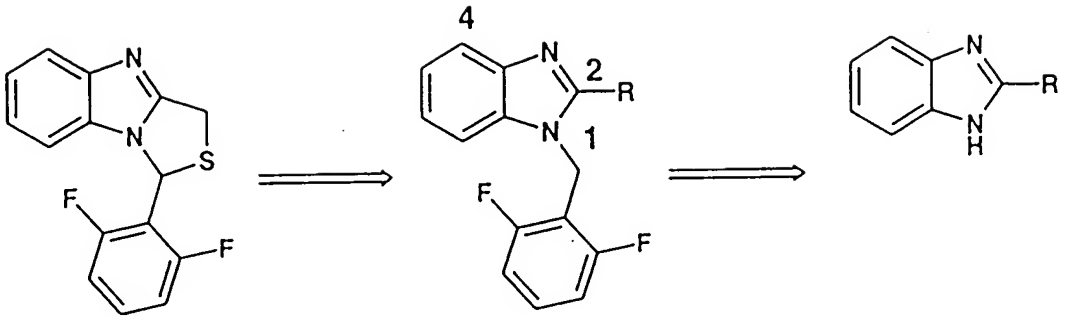


IMIDAZOLE



BENZIMIDAZOLE

FIGURE 2



TBZ

FIGURE 3

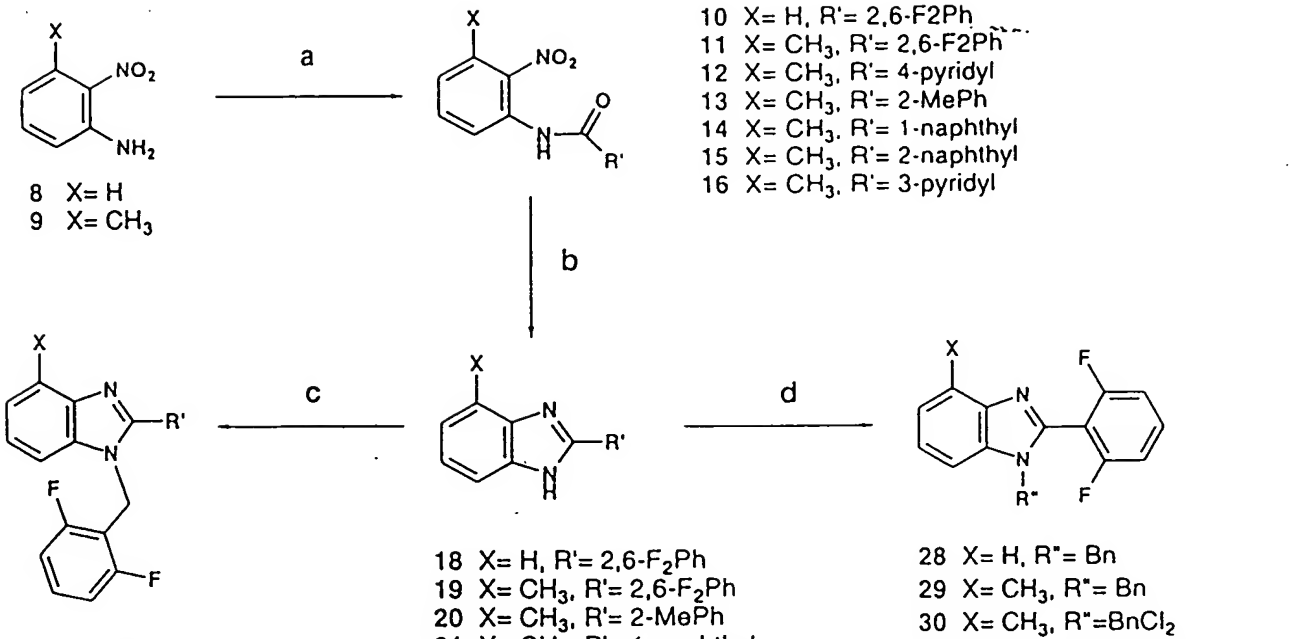

X
NO2
NH2
a
X
NO2
O
N
H
R'
10 X= H, R'= 2,6-F2Ph
11 X= CH3, R'= 2,6-F2Ph
12 X= CH3, R'= 4-pyridyl
13 X= CH3, R'= 2-MePh
14 X= CH3, R'= 1-naphthyl
15 X= CH3, R'= 2-naphthyl
16 X= CH3, R'= 3-pyridyl
8 X= H
9 X= CH3
b
X
N
R'
N
F
F
c
X
N
R'
N
H
d
X
F
N
R'
N
R"
F
26 X= H, R'= 2,6-F2Ph
33 X= CH3, R'= 2,6-F2Ph
36 X= H, R'= 2-MePh
37 X= CH3, R'= 1-naphthyl
38 X= CH3, R'= 2-naphthyl
40 X= CH3, R'= 3-pyridyl
41 X= CH3, R'= 4-pyridyl
18 X= H, R'= 2,6-F2Ph
19 X= CH3, R'= 2,6-F2Ph
20 X= CH3, R'= 2-MePh
21 X= CH3, R'= 1-naphthyl
22 X= CH3, R'= 2-naphthyl
23 X= CH3, R'= 3-pyridyl
24 X= CH3, R'= 4-pyridyl
28 X= H, R"= Bn
29 X= CH3, R"= Bn
30 X= CH3, R"=BnCl2
42 X= CH3, R"= 2,3,4,5,6-F5Ph
43 X= CH3, R"= 3-pyridyl
45 X= H, R"=PhSO2
46 X= CH3, R"=PhSO2
47 X= H, R"=2,6-F2Bz

FIGURE 4

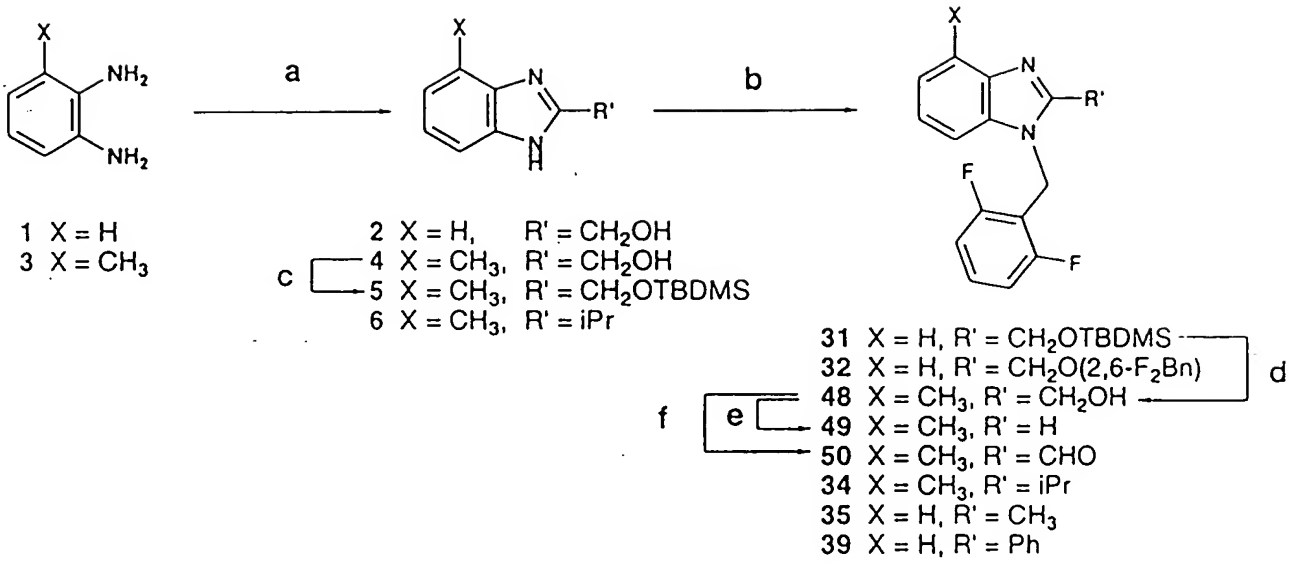
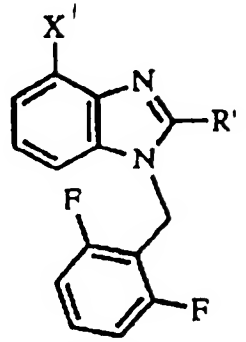
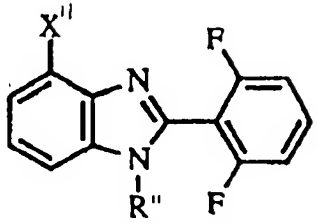

X
NH2
NH2
a
X
N
R'
N
H
b
X
N
R'
N
F
F
1 X = H
3 X = CH3
c
2 X = H, R' = CH2OH
4 X = CH3, R' = CH2OH
5 X = CH3, R' = CH2OTBDMS
6 X = CH3, R' = iPr
31 X = H, R' = CH2OTBDMS
32 X = H, R' = CH2O(2,6-F2Bn)
48 X = CH3, R' = CH2OH
d
f
e
49 X = CH3, R' = H
50 X = CH3, R' = CHO
34 X = CH3, R' = iPr
35 X = H, R' = CH3
39 X = H, R' = Ph

FIGURE 5



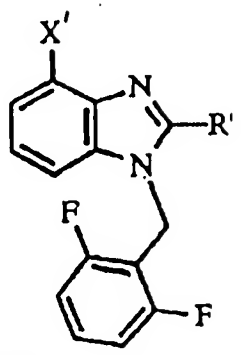
No.	X'	R'	formula	mp, °C	anal.	% inhibition (10 μM)	IC_{50}(μM)
39	H	Ph	$C_{20}H_{14}F_2N_2$	127-129	C,H,N	77	1.7
26	H	2,6-F_2Ph	$C_{20}H_{12}F_4N_2$	138-140	C,H,N	93	0.4
33	CH_3	2,6-F_2Ph	$C_{21}H_{14}F_4N_2$	182-186	C,H,N	92	0.2
36	H	2-CH_3-Ph	$C_{21}H_{16}F_2N_2$	138-140	C,H,N	71	3.5
41	CH_3	4-Py	$C_{20}H_{15}F_2N_3$	171-172	C,H,N	70	2.2
40	CH_3	3-Py	$C_{20}H_{15}F_2N_3$	186-188	C,H,N	35	
37	CH_3	1-Nap	$C_{25}H_{18}F_2N_2$	121-123	C,H,N	2	
38	CH_3	2-Nap	$C_{25}H_{18}F_2N_2$	175-176	C,H,N	8	
8-Cl-TIBO			$C_{16}H_{20}ClN_3xHCl$			99	0.02
TBZ			$C_{13}H_{10}F_2N_2S$			84	1.0

FIGURE 6



No.	X^{II}	R''	formula	mp, °C	anal.	% inhibition (10 μM)	IC_{50}(μM)
26	H	2,6-F_2Bn	$C_{20}H_{12}F_4N_2$	138-140	C,H,N	93	0.4
33	CH_3	2,6-F_2Bn	$C_{21}H_{14}F_4N_2$	182-186	C,H,N	92	0.2
28	H	Bn	$C_{20}H_{14}F_2N_2$	122-125	C,H,N	71	3.4
29	CH_3	Bn	$C_{21}H_{16}F_2N_2$	112-117	C,H,N[a]	87	1.3
30	CH_3	2,6-Cl_2Bn	$C_{21}H_{14}Cl_2F_2N_2$	202-203	C,H,N	58	
42	CH_3	2,3,4,5,6-F_5Bn	$C_{21}H_{11}F_7N_2$	155-156	C,H,N	36	
43	CH_3	CH_2(3-Py)	$C_{20}H_{15}F_2N_3$	131-132	C,H,N	43	
45	H	$PhSO_2$	$C_{19}H_{12}F_2N_2SO_2$	104-106	C,H,N	52	
46	CH_3	$PhSO_2$	$C_{20}H_{14}F_2N_2SO_2$	134-135	C,H,N	39	
47	H	2,6-F_2Bz	$C_{20}H_{10}F_4N_2O$	144-146	C,H,N	8	

FIGURE 7

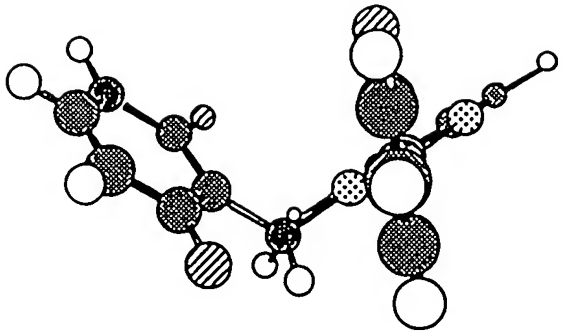


No.	X'	R'	formula	mp, °C	anal.	% inhibition (10 μM)[a]
39	H	Ph	$C_{20}H_{14}F_2N_2$	127-129	C,H,N	77
50	CH_3	CHO	$C_{16}H_{12}F_2N_2O$	144-146	C,H,N	59
34	CH_3	iPr	$C_{18}H_{18}F_2N_2$	151-153	C,H,N	53
49	CH_3	H	$C_{15}H_{12}F_2N_2$	98-100	C,H,N	40
35	H	CH_3	$C_{15}H_{12}F_2N_2$	99-100	C,H,N	22
48	CH_3	CH_2OH	$C_{16}H_{14}F_2N_2O$	203-205	C,H,N	12
32	H	CH_2O(2,6-F_2Bn)	$C_{22}H_{16}F_4N_2O$	107-109	C,H,N	7

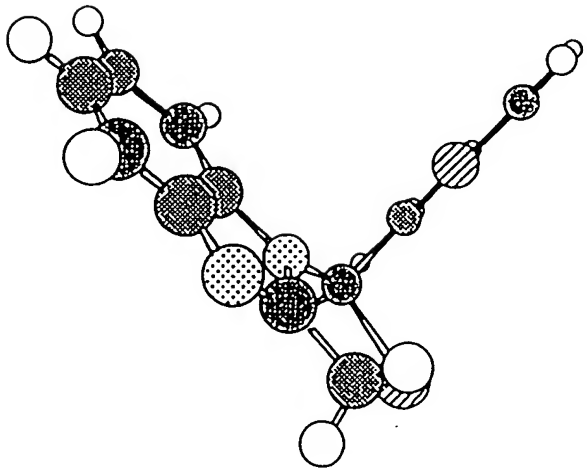
FIGURE 8

Isolate	33	26	TBZ	TIBO
NL4-3 (WT)	0.5	1.85	1.7	0.3
L74V	0.1	0.46	0.7	0.2
A98G	1.4	4.75	17.7	11
L100I	0.3	1.36	12.2	17.4
K101E	16.7	>20	>20	17.4
K103N	8.1	12.9	>20	17.4
V106A	20	>20	>20	12.5
V108I	2.8	10.4	9.7	2.4
V179D	0.5	2.3	3.1	6.2
Y181C	6	15.2	16.3	4.2
Y188C	2.3	11.7	>20	>17.4
4xAZT	0.1	0.27	1.5	0.3
4xAZT/L100I	0.2	0.84	1.7	>17.4
4xAZT/Y181C	3.5	>20	14.5	2.0

FIGURE 9

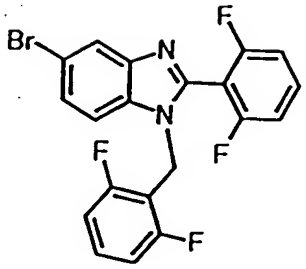


39

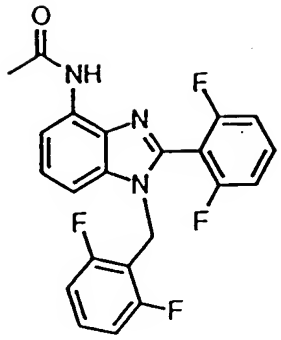


TBZ

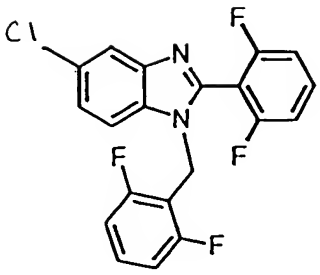
FIGURE 10



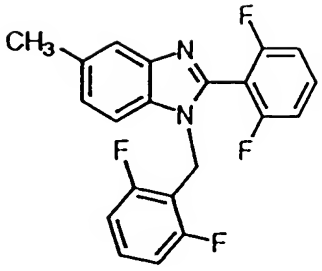
(2100)



(3600)



(2200)



(2400)

FIGURE 11

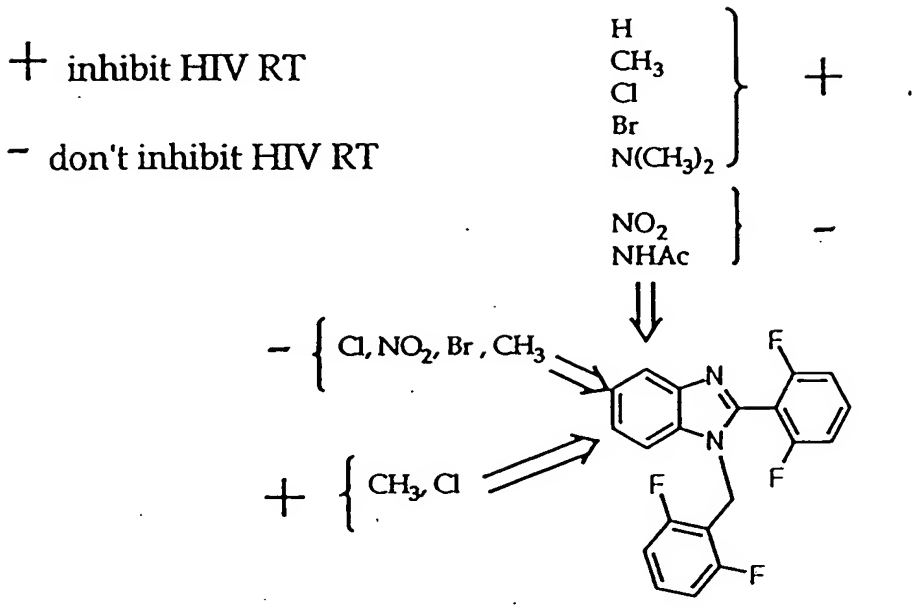

+ inhibit HIV RT
- don't inhibit HIV RT
H
CH3
Cl
Br
N(CH3)2
+
NO2
NHAc
-
- { Cl, NO2, Br , CH3
+ { CH3, Cl
N
N
F
F
F
F

FIGURE 12

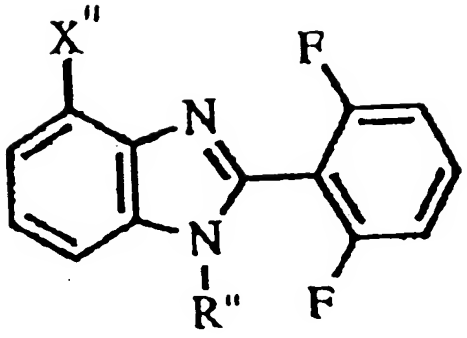


FIGURE 13

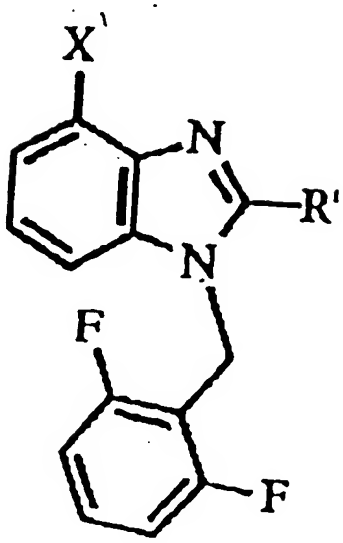


FIGURE 14

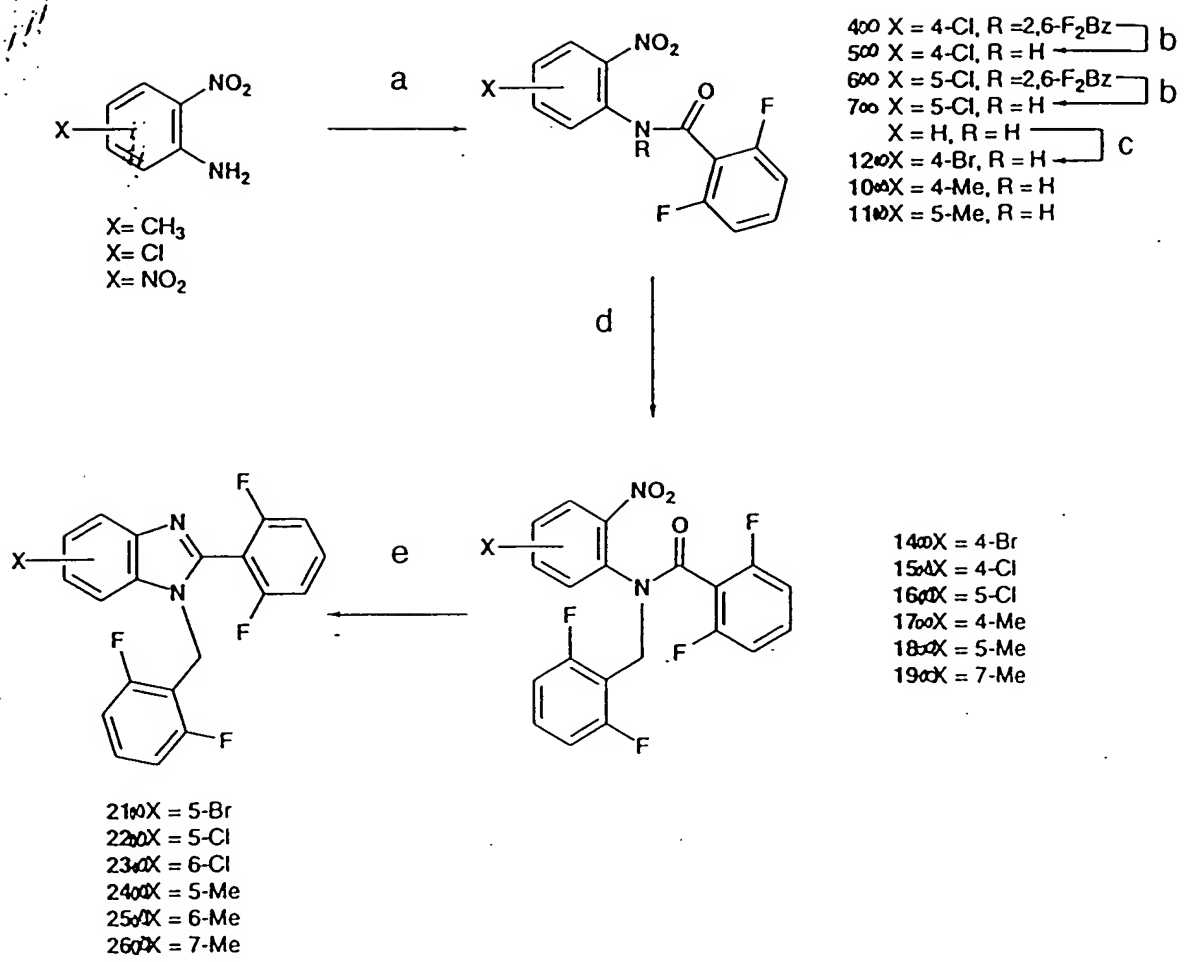

a
b
c
d
e
X= CH3
X= Cl
X= NO2
400 X = 4-Cl, R =2,6-F2Bz
500 X = 4-Cl, R = H
600 X = 5-Cl, R =2,6-F2Bz
700 X = 5-Cl, R = H
X = H, R = H
1200X = 4-Br, R = H
1000X = 4-Me, R = H
1100X = 5-Me, R = H
1400X = 4-Br
1500X = 4-Cl
1600X = 5-Cl
1700X = 4-Me
1800X = 5-Me
1900X = 7-Me
2100X = 5-Br
2200X = 5-Cl
2300X = 6-Cl
2400X = 5-Me
2500X = 6-Me
2600X = 7-Me

FIGURE 15

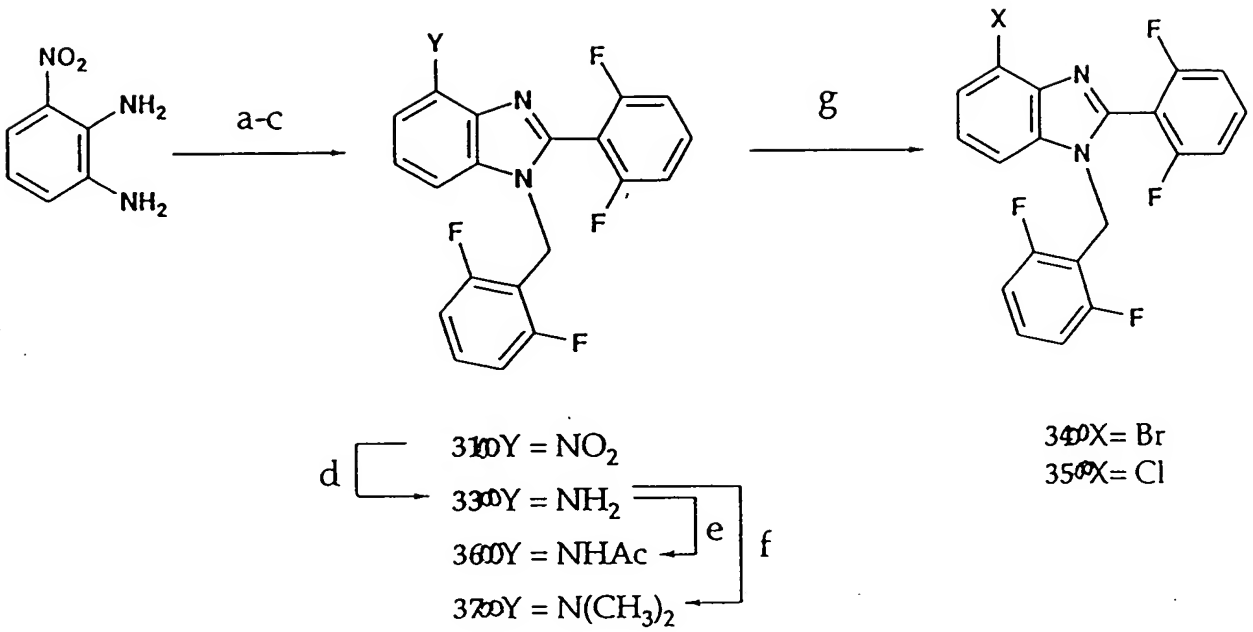
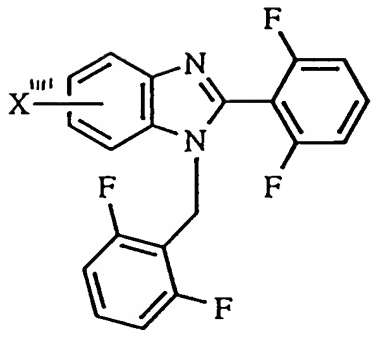

NO2
NH2
NH2
a-c
Y
N
N
F
F
F
F
g
X
N
N
F
F
F
F
d
310 Y = NO2
330 Y = NH2
360 Y = NHAc
370 Y = N(CH3)2
e
f
340 X= Br
350 X= Cl

FIGURE 16

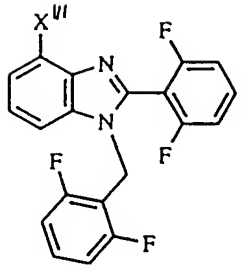


No.	X'''	formula	mp, °C	anal.	% inhibition (1 μM)
200	H	$C_{20}H_{12}F_4N_2$	138-140	C,H,N	58
100	4-CH_3	$C_{21}H_{14}F_4N_2$	182-186	C,H,N	71
2400	5-CH_3	$C_{21}H_{14}F_4N_2$	147-149	C,H,N	27
2500	6-CH_3	$C_{21}H_{14}F_4N_2$	172-173	C,H,N	51
2600	7-CH_3	$C_{21}H_{14}F_4N_2$	177-178	C,H,N	2
2800	4,5-CH_3	$C_{22}H_{16}F_4N_2$	176-177	C,H,N	36

FIGURE 17

No.	X	formula	mp, °C	anal.	% inhibition (1 μM)
3500	4-Cl	$C_{20}H_{11}ClF_4N_2$	163-164	C,H,N	75
2200	5-Cl	$C_{20}H_{11}ClF_4N_2$	136-137	C,H,N	48
2300	6-Cl	$C_{20}H_{11}ClF_4N_2$	152-153	C,H,N	69
3400	4-Br	$C_{20}H_{11}BrF_4N_2$	147-148	C,H,N	78
2100	5-Br	$C_{20}H_{11}BrF_4N_2$	155-156	C,H,N	43
3100	4-NO_2	$C_{20}H_{11}F_4N_3O_2$	168-170	C,H,N	51
2900	5-NO_2	$C_{20}H_{11}F_4N_3O_2$	168-169	C,H,N	30

FIGURE 18



No.	X[III]	formula	mp, °C	anal.	% inhibition (1 μM)	IC_{50} (μM)
100	CH_3	$C_{21}H_{14}F_4N_2$	182-186	C,H,N	71	0.8
3300	NH_2	$C_{20}H_{13}F_4N_3$	168-170	C,H,N	69	1.1
3600	NHAc	$C_{22}H_{15}F_4N_3O$	194-195	C,H,N	24	
3700	$N(CH_3)_2$	$C_{22}H_{17}F_4N_3$	155-156	C,H,N	77	2.0
3400	Br	$C_{20}H_{11}BrF_4N_2$	147-148	C,H,N	78	1.1
3500	Cl	$C_{20}H_{11}ClF_4N_2$	163-164	C,H,N	75	1.4
8-Cl-TIBO		$C_{16}H_{20}ClN_3 x HCl$		C,H,N	98	0.06
TBZ		$C_{15}H_{10}F_2N_2S$		C,H,N	57	1.4

FIGURE 19

SUBSTITUENT IN POSITION 4

Isolate	CH_3	NH_2	Cl	Br	8-Cl TIBO	NVP
NL4-3 (WT)	0.5	0.4	0.5	0.5	0.3	0.04
L74V	0.1	0.1	0.5	0.1	0.2	<0.02
A98G	1.4	1.3	3.5	1.1	11	0.8
L100I	0.3	1.2	0.3	0.5	17.4	0.4
K101E	16.7	> 20	> 20	> 20	17.4	1.1
K103N	8.1	3.1	10.2	> 20	17.4	>7.5
V106A	20	> 20	14.4	> 20	12.5	>7.5
V108I	2.8	3.3	3.2	4.2	2.4	0.2
V179D	0.5	0.3	0.6	0.8	6.2	0.1
Y181C	6	9.2	8.4	10.7	4.2	4.9
Y188C	2.3	2	3.4	3.7	>17.4	ND
4xAZT	0.1	0.2	0.2	0.3	0.3	ND
4xAZT /L100I	0.2	0.4	0.3	0.3	>17.4	0.04
4xAZT /Y181C	3.5	11.4	>20	20	2.0	0.1

FIGURE 20

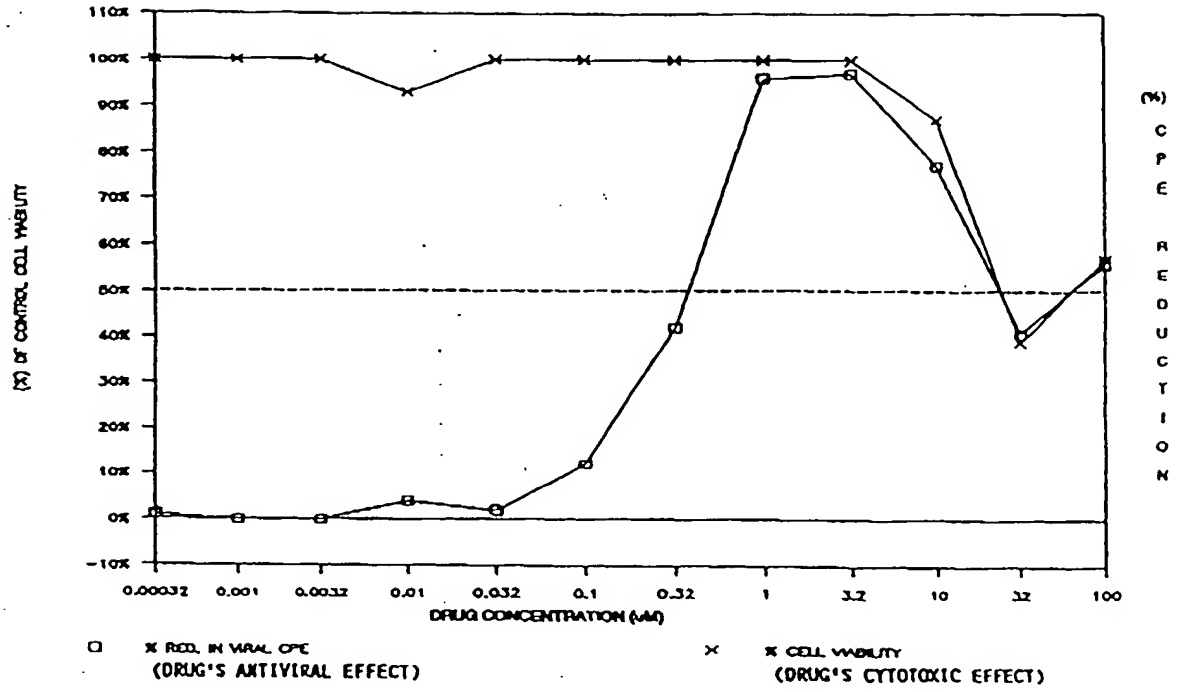

110%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
-10%
(%) OF CONTROL CELL VIABILITY
(%) CPE REDUCTION
0.00032
0.001
0.0032
0.01
0.032
0.1
0.32
1
3.2
10
32
100
DRUG CONCENTRATION (uM)
% RED. IN VIRAL CPE
(DRUG'S ANTIVIRAL EFFECT)
% CELL VIABILITY
(DRUG'S CYTOTOXIC EFFECT)

FIGURE 21

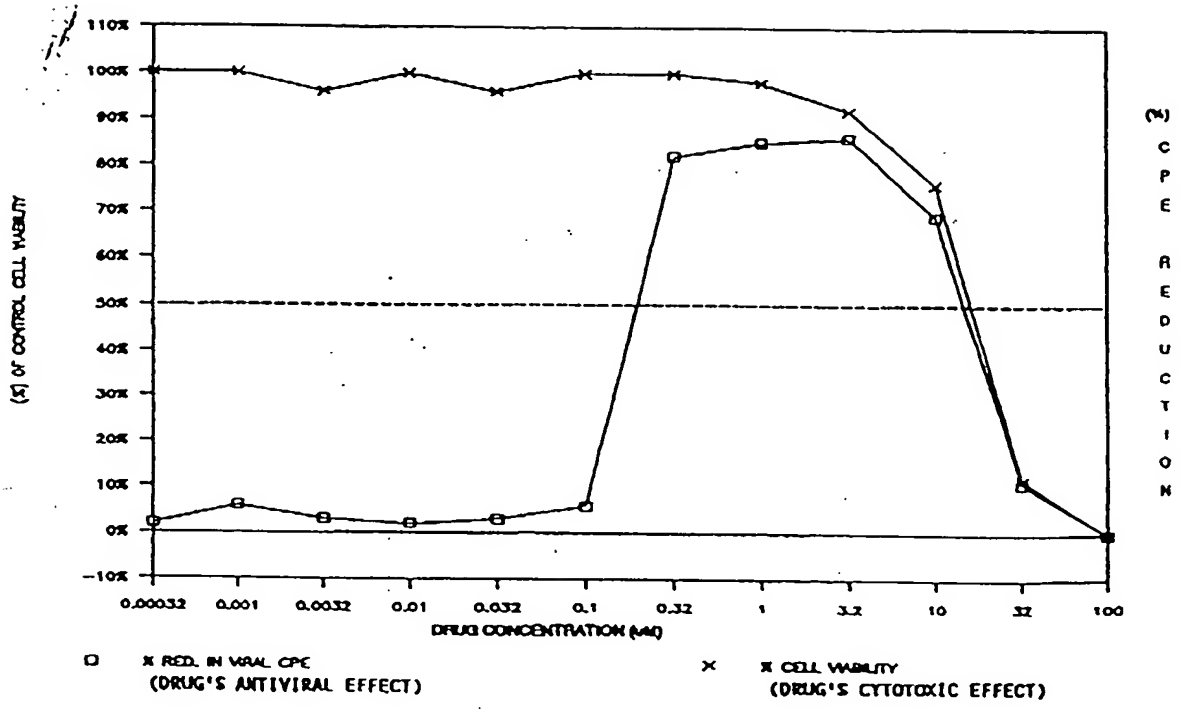

110%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
-10%
(%) OF CONTROL CELL VIABILITY
(%) CPE REDUCTION
0.00032
0.001
0.0032
0.01
0.032
0.1
0.32
1
3.2
10
32
100
DRUG CONCENTRATION (uM)
% RED. IN VIRAL CPE
(DRUG'S ANTIVIRAL EFFECT)
% CELL VIABILITY
(DRUG'S CYTOTOXIC EFFECT)

FIGURE 22

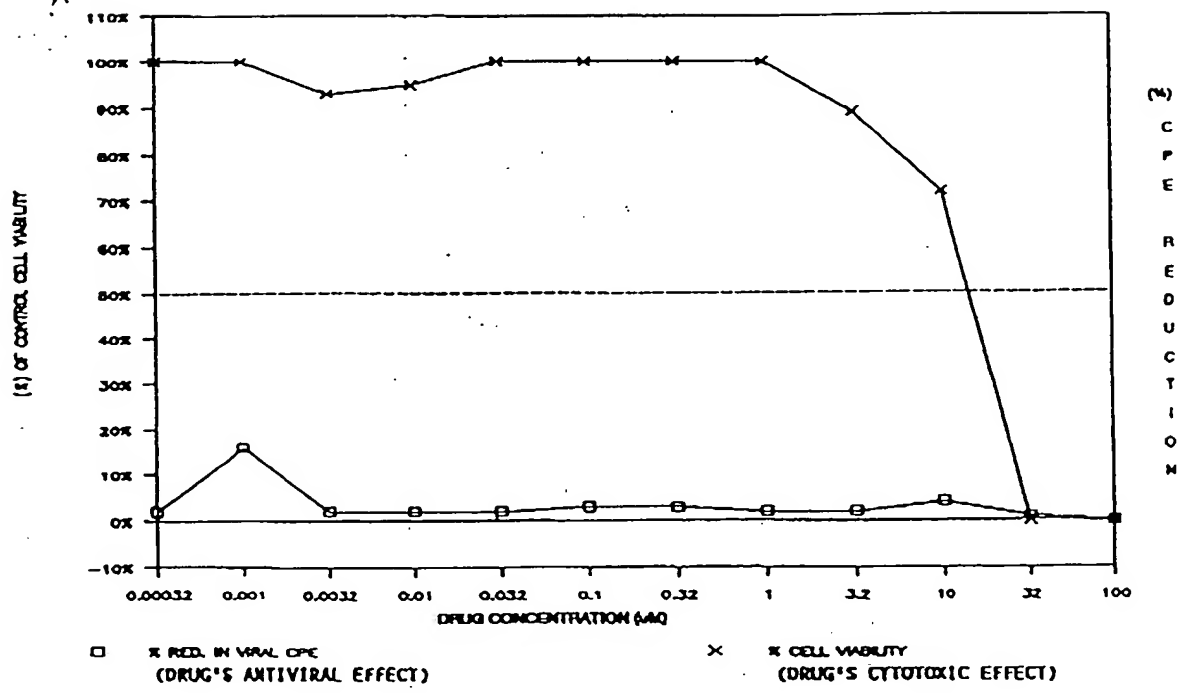

(%) OF CONTROL CELL VIABILITY
110%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
-10%
0.00032
0.001
0.0032
0.01
0.032
0.1
0.32
1
3.2
10
32
100
DRUG CONCENTRATION (uM)
(%) CPE REDUCTION
% RED. IN VIRAL CPE
(DRUG'S ANTIVIRAL EFFECT)
% CELL VIABILITY
(DRUG'S CYTOTOXIC EFFECT)

FIGURE 23

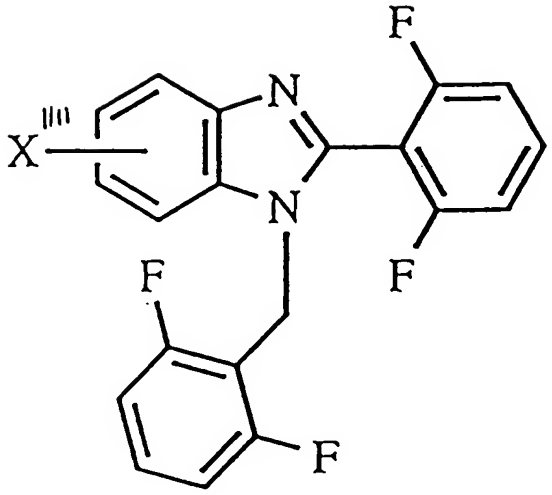


FIGURE 24

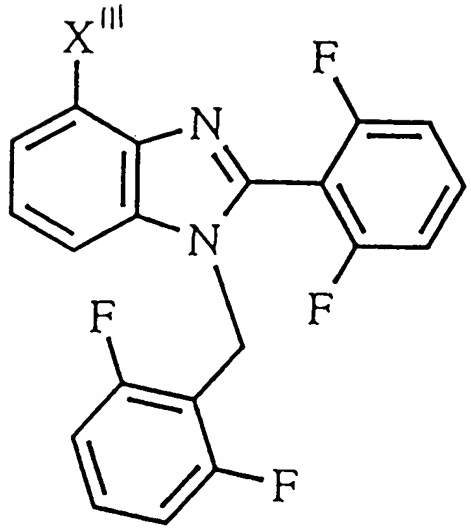


FIGURE 25

No	formula	calculated			found		
		C	H	N	C	H	N
26	$C_{20}H_{12}F_4N_2$	67.42	3.39	7.86	67.28	3.44	7.76
28	$C_{20}H_{14}F_2N_2 x 1/8H_2O$	74.47	4.45	8.68	74.43	4.45	8.63
29	$C_{21}H_{16}F_2N_2 x 1/4H_2O$	74.43	4.91	8.27	74.81	4.90	7.85
30	$C_{21}H_{14}Cl_2F_2N_2 x 1/4H_2O$	61.86	3.58	6.87	61.64	3.65	6.80
32	$C_{22}H_{16}F_4N_2O x 1/2H_2O$	64.55	4.19	6.84	64.63	4.11	6.72
33	$C_{21}H_{14}F_4N_2$	68.11	3.81	7.56	68.17	3.90	7.54
34	$C_{18}H_{18}F_2N_2$	71.98	6.04	9.33	72.06	6.05	9.25
35	$C_{15}H_{12}F_2N_2$	69.76	4.68	10.85	69.56	4.77	10.79
36	$C_{21}H_{16}F_2N_2$	75.44	4.82	8.38	75.16	4.89	8.29
37	$C_{25}H_{18}F_2N_2$	78.11	4.72	7.29	78.00	4.75	7.19
38	$C_{25}H_{18}F_2N_2$	78.11	4.72	7.29	77.84	4.83	7.23
39	$C_{20}H_{14}F_2N_2 x 1/4H_2O$	73.95	4.50	8.62	73.87	4.50	8.60
40	$C_{20}H_{15}F_2N_3$	71.63	4.51	12.53	71.74	4.55	12.62
41	$C_{20}H_{15}F_2N_3$	71.63	4.51	12.53	71.72	4.55	12.58
42	$C_{21}H_{11}F_7N_2$	59.44	2.61	6.60	59.35	2.65	6.50
43	$C_{20}H_{15}F_2N_3 x 1/2H_2O$	69.76	4.68	12.20	69.59	4.38	12.29
45	$C_{19}H_{12}F_2N_2SO_2$	61.62	3.27	7.56	61.68	3.34	7.60
46	$C_{20}H_{14}F_2N_2SO_2$	62.49	3.67	7.29	62.50	3.71	7.24
47	$C_{20}H_{10}F_4N_2O$	64.87	2.72	7.56	64.88	2.77	7.46
48	$C_{16}H_{14}F_2N_2O$	66.66	4.89	9.72	66.74	4.94	9.71
49	$C_{15}H_{12}F_2N_2 x 1/4H_2O$	68.56	4.79	10.66	68.52	4.60	10.75
50	$C_{16}H_{12}F_2N_2O x 1/5H_2O$	66.29	4.31	9.66	66.57	4.28	9.67

No.	formula	calculated			found		
		C	H	N	C	H	N
3100	$C_{20}H_{13}F_4N_3$	64.69	3.53	11.32	64.63	3.54	11.20
3400	$C_{22}H_{15}F_4N_3O$	63.92	3.66	10.17	63.94	3.62	10.10
3500	$C_{22}H_{17}F_4N_3$	66.16	4.29	10.52	66.24	4.24	10.47
3200	$C_{20}H_{11}BrF_4N_2 x 1/4H_2O$	54.63	2.64	6.37	54.59	2.47	6.38
1800	$C_{20}H_{11}BrF_4N_2 x 3/4H_2O$	53.53	2.81	6.24	53.23	2.49	6.10
3300	$C_{20}H_{11}ClF_4N_2$	61.47	2.84	7.17	61.59	2.86	7.23
2000	$C_{20}H_{11}ClF_4N_2$	61.47	2.84	7.17	61.37	2.90	7.15
2100	$C_{20}H_{11}ClF_4N_2$	61.47	2.84	7.17	61.31	2.92	7.04
2200	$C_{21}H_{14}F_4N_2$	68.11	3.81	7.56	68.22	3.90	7.60
2300	$C_{21}H_{14}F_4N_2$	68.11	3.81	7.56	67.94	3.86	7.50
2400	$C_{21}H_{14}F_4N_2$	68.11	3.81	7.56	68.02	3.89	7.49
2800	$C_{22}H_{16}F_4N_2$	68.75	4.20	7.29	68.68	4.15	7.28
2900	$C_{20}H_{11}F_4N_3O_2$	59.86	2.76	10.47	59.96	2.78	10.55
3000	$C_{20}H_{11}F_4N_3O_2$	59.86	2.76	10.47	60.24	2.89	10.38